THE JOURNAL OF PHYSIOLOGY

EDITED FOR

THE PHYSIOLOGICAL SOCIETY

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VOL. 88

1937
1936-37

CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4

74/18
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PRINTED IN GREAT BRITAIN

9504

CONTENTS OF VOL. 88

No. 1. 16 *October* 1936

PAGE

The action of antidromic impulses on ganglion cells. By J. C. Eccles 1

Carbamino compounds of CO_2 with human hæmoglobin and their role in the transport of CO_2. By J. K. W. Ferguson . . 40

The genesis of respiratory movements in the fœtus of the sheep. By J. Barcroft and D. H. Barron 56

Dietary deficiency, nerve lesions and the dental tissues. By J. D. King 62

The double motor innervation of the adductor muscle in the claw of the crayfish. By A. van Harreveld and C. A. G. Wiersma 78

The action of progesterone on the uterus of the rabbit and its antagonism by œstrone. By J. M. Robson 100

No. 2. 6 *November* 1936

Mechanism of the peripheral vascular responses to changes in blood gas tension in man. By B. Bolton, E. A. Carmichael and D. J. Williams 113

The spread of activity in the cerebral cortex. By E. D. Adrian 127

Alkaline resistance and spreading velocity of fœtal and adult types of mammalian hæmoglobin. By R. Brinkman and J. H. P. Jonxis 162

Hue discrimination and its relation to the adaptation of the eye. By W. D. Wright 167

Hue discrimination in normal and abnormal colour vision. By H. V. Corbett 176

The effect of adrenaline on muscle glycogen in adrenalectomized, thyroidectomized, and hypophysectomized rats. By J. B. Collip, D. L. Thomson and G. Toby 191

Contralateral effects from tension receptors in skeletal muscle. By Stuart Morson and Gilbert Phillips 199

PAGE

On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandid and vesiglandin). By U. S. von Euler . . . 213

The technique of suprarenalectomy in the guinea-pig and the survival period and cause of death of guinea-pigs after bilateral suprarenalectomy. By H. T. Simmons and Raymond Whitehead 235

Multiple response to constant current in frog's medullated nerve. By Bernhard Katz 239

No. 3. 11 *December* 1936

Dilatation of veins in response to tapping in man and in certain other mammals. By K. J. Franklin and A. D. McLachlin . 257

The reactions of the abdominal vena cava. By K. J. Franklin and A. D. McLachlin 261

Stream lines in the abdominal vena cava in the late stages of pregnancy. By K. J. Franklin and A. D. McLachlin . . . 263

The acetylcholine metabolism of a sympathetic ganglion. By G. L. Brown and W. Feldberg 265

Reflex slowing of respiration accompanying changes in the intrapulmonary pressure. By M. Hammouda and W. H. Wilson . 284

Species variation in thyrotropic activity of the pituitary gland. By I. W. Rowlands 298

Inhibition of ovulation in the rabbit by anti-gonadotropic serum. By A. S. Parkes and I. W. Rowlands 305

The effect of certain hormones on the activity of the uterine muscle of the guinea-pig. By G. H. Bell and J. M. Robson . 312

Experiments on the mechanism of strychnine "curarization". By H. D. Bouman 328

Sympathetic vaso-dilator fibres in the hare and the monkey compared with other species. By Edith Bülbring and J. H. Burn 341

The induction of pseudo-pregnancy in the rat by electrical stimulation through the head. By G. W. Harris 361

No. 4. 18 *January* 1937

PAGE

Water metabolism in relation to the menstrual cycle. By P. L. KROHN and S. ZUCKERMAN 369

"Sensitization" by injury of the cutaneous nerve endings in the frog. By FRANCIS ECHLIN and NICOLAY PROPPER 388

The relation of the pituitary to liver glycogen production and utilization. By OLIVER COPE 401

Adrenaline and the blood lactic acid level in hypophysectomized rabbits. By OLIVER COPE and RICHARD H. THOMPSON 417

The study of the carotid sinus respiratory reflexes by means of chronic experiments. By DAVID H. SMYTH 425

The electrical responses of dark-adapted frogs' eyes to monochromatic stimuli. By R. GRANIT and A. MUNSTERHJELM . . 436

Effects on the knee jerk of stimulation of the central end of the vagus and of various changes in the circulation and respiration. By A. SCHWEITZER and SAMSON WRIGHT 459

The action of adrenaline on the knee jerk. By A. SCHWEITZER and SAMSON WRIGHT 476

The changes in viscosity of an unstriated muscle (*Mytilus edulis*) during and after stimulation with alternating, interrupted and uninterrupted direct currents. By F. R. WINTON 492

LIST OF AUTHORS

PAGE

ADRIAN, E. D. Spread of activity in cerebral cortex 127
BARCROFT, J. and BARRON, D. H. Respiratory movements in fœtus of sheep 56
BARRON, D. H. and BARCROFT, J. Respiratory movements in fœtus of sheep 56
BELL, G. H. Hormones on uterus of guinea-pig 312
BOLTON, B., CARMICHAEL, E. ARNOLD and WILLIAMS, D. J. Blood gases and vaso-motor changes in man 113
BOUMAN, H. D. Mechanism of strychnine "curarization" 328
BRINKMAN, R. and JONXIS, J. H. P. Hæmoglobins in blood . . 162
BROWN, G. L. and FELDBERG, W. Acetylcholine output from ganglion 265
BÜLBRING, EDITH and BURN, J. H. Sympathetic vaso-dilator fibres . 341
BURN, J. H. and BÜLBRING, EDITH. Sympathetic vaso-dilator fibres . 341
CARMICHAEL, E. ARNOLD, WILLIAMS, D. J. and BOLTON, B. Blood gases and vaso-motor changes in man 113
COLLIP, J. B., THOMSON, D. L. and TOBY, G. Adrenaline and muscle glycogen 191
COPE, OLIVER. Pituitary and liver glycogen 401
COPE, OLIVER and THOMPSON, RICHARD H. Pituitary, adrenaline and blood lactate 417
CORBETT, H. V. Hue discrimination 176
ECCLES, J. C. Antidromic impulses 1
ECHLIN, FRANCIS and PROPPER, NICOLAY. Cutaneous nerve endings in the frog 388
EULER, U. S. VON. Prostaglandin and vesiglandin 213
FELDBERG, W. and BROWN, G. L. Acetylcholine output from ganglion 265
FERGUSON, J. K. W. Carbamino compounds of human hæmoglobin . 40
FRANKLIN, K. J. and MCLACHLIN, A. D. Dilatation of veins on tapping 257
FRANKLIN, K. J. and MCLACHLIN, A. D. Reactions of abdominal vena cava 261
FRANKLIN, K. J. and MCLACHLIN, A. D. Stream lines in abdominal vena cava 263
GRANIT, R. and MUNSTERHJELM, A. Electrical response of frog's eye . 436
HAMMOUDA, M. and WILSON, W. H. Respiration and intrapulmonary pressure 284
HARREVELD, A. VAN and WIERSMA, C. A. G. Double motor innervation of crayfish 78
HARRIS, G. W. Pseudo-pregnancy in the rat 361
JONXIS, J. H. P. and BRINKMAN, R. Hæmoglobins in blood . . 162
KATZ, BERNHARD. Multiple response to constant current 239
KING, J. D. Vitamin and dental nerves 62
KROHN, P. L. and ZUCKERMAN, S. Water metabolism and menstrual cycle 389

PAGE

McLachlin, A. D. and Franklin, K. J. Dilatation of veins on tapping 257
McLachlin, A. D. and Franklin, K. J. Reactions of abdominal vena cava 261
McLachlin, A. D. and Franklin, K. J. Stream lines in abdominal vena cava 263
Morson, Stuart and Phillips, Gilbert. Tension receptors in skeletal muscle 199
Munsterhjelm, A. and Granit, R. Electrical response of frog's eye . 436
Parkes, A. S. and Rowlands, I. W. Anti-gonadotropic serum . . 305
Phillips, Gilbert and Morson, Stuart. Tension receptors in skeletal muscle 199
Proffer, Nicolay and Echlin, Francis. Cutaneous nerve endings in the frog 388
Robson, J. M. Progesterone and œstrone 100
Robson, J. M. and Bell, G. H. Hormones on uterus of guinea-pig . 312
Rowlands, I. W. Thyrotropic activity 298
Rowlands, I. W. and Parkes, A. S. Anti-gonadotropic serum . . 305
Schweitzer, A. and Wright, Samson. Autonomic effects on knee jerk 459
Schweitzer, A. and Wright, Samson. Action of adrenaline on the knee jerk 476
Simmons, H. T. and Whitehead, Raymond. Suprarenalectomy in guinea-pigs 235
Smyth, David H. Carotid sinus and respiration 425
Thompson, Richard H. and Cope, Oliver. Pituitary, adrenaline and blood lactate 417
Thomson, D. L., Toby, G. and Collip, J. B. Adrenaline and muscle glycogen 191
Toby, G., Collip, J. B. and Thomson, D. L. Adrenaline and muscle glycogen 191
Whitehead, Raymond and Simmons, H. T. Suprarenalectomy in guinea-pigs 235
Wiersma, C. A. G. and Harreveld, A. van. Double motor innervation of crayfish 78
Williams, D. J., Bolton, B. and Carmichael, E. Arnold. Blood gases and vaso-motor changes in man 113
Wilson, W. H. and Hammouda, M. Respiration and intrapulmonary pressure 284
Winton, F. R. Changes in viscosity of unstriated muscle 492
Wright, Samson and Schweitzer, A. Autonomic effects on knee jerk 459
Wright, Samson and Schweitzer, A. Action of adrenaline on the knee jerk 476
Wright, W. D. Hue discrimination 167
Zuckerman, S. and Krohn, P. L. Water metabolism and menstrual cycle 369

PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY

17 *October* 1936

PAGE

Kane, F. and O'Connor, J. M. The relation between the *p*H of the blood and the law of isodynamic equivalence 1*P*

Burns, D. and Secker, J. Potentiation of adrenaline activity . . 2*P*

Cowan, S. L. The initiation of all-or-none responses in muscle by acetylcholine 3*P*

Raventos, J. Synergism and antagonism of acetylcholine and quaternary ammonium compounds 5*P*

Dyer, F. J. Standardization of prolactin 6*P*

14 *November* 1936

Reid, Charles. Correlation of observations on the sugar production of the livers of normal and diabetic fasting dogs 9*P*

Code, C. F. The source of the histamine-like constituent of blood . 10*P*

McDowall, R. J. S. and McWhan, I. Adrenaline dilatation . . 11*P*

LIST OF AUTHORS

Burns, D. and Secker, J. Potentiation of adrenaline activity . . 2*P*

Code, C. F. The histamine-like constituent of blood 10*P*

Cowan, S. L. All-or-none responses in muscle with acetylcholine . 3*P*

Dyer, F. J. Standardization of prolactin 6*P*

Kane, F. and O'Connor, J. M. Blood *p*H and law of isodynamic equivalence 1*P*

McDowall, R. J. S. and McWhan, I. Adrenaline dilatation . . 11*P*

McWhan, I. and McDowall, R. J. S. Adrenaline dilatation . . 11*P*

O'Connor, J. M. and Kane, F. Blood *p*H and law of isodynamic equivalence 1*P*

Raventos, J. Effects of acetylcholine and quaternary ammonium compounds 5*P*

Reid, Charles. Sugar production of livers of normal and diabetic fasting dogs 9*P*

Secker, J. and Burns, D. Potentiation of adrenaline activity . . 2*P*

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THE ACTIONS OF ANTIDROMIC IMPULSES ON GANGLION CELLS

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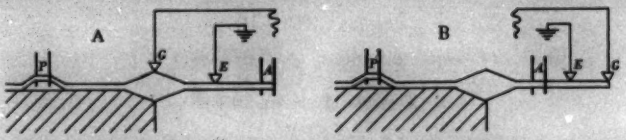
(*Received April* 15, 1936)

ANTIDROMIC impulses backfired into motoneurones have been employed in many recent investigations on motoneurones [Denny Brown, 1928, 1929; Eccles, 1931; Eccles & Sherrington, 1931*b*, *c*; Eccles & Hoff, 1932; Gasser & Graham, 1933; Umrath, 1933; Hughes & Gasser, 1934*a*; Lorente de Nó, 1935*b*], but complications introduced by the uncertain extent to which motoneurones are played upon by internuncial neurones have always presented difficulties in the interpretation of the experiments. On account of the absence of these internuncial neurones the superior cervical ganglion [cf. Brown, 1934] is particularly suitable for this attempt to reinvestigate the action of an antidromic impulse on a nerve cell, a subject on which there is much conflict of opinion [cf. Lorente de Nó, 1935*b*]. A preliminary account of this work has already been published [Eccles, 1935*d*].

The method of experiment is similar to that previously described [Eccles, 1935*a*], but, on account of the frequent shortness of the postganglionic trunk in cats, Belgian hares have been employed in five out of the thirty experiments. With the exception of the non-medullated S_1 postganglionic fibres [Bishop & Heinbecker, 1932], the S_1 and S_2 groups of ganglion cells correspond with those in cats, so no special reference need be made to the experiments on Belgian hares. In all experiments antidromic volleys in the postganglionic trunk have been set up by induction shocks applied through electrodes placed on this trunk well clear of the ganglion, and the responses of the ganglion cells have been recorded by electrodes *G* and *E* placed in either of the positions of Text-fig. 1. In position A the electrical responses of the ganglion cells to preganglionic or antidromic volleys are recorded directly, while in position B these responses are only indirectly observed, appearing as changes in the

impulses discharged by the ganglion cells in response to a preganglionic volley set up at electrodes *P*. This latter method of recording was only used in some of the experiments of section D. Stimulus artefacts are diminished by carefully stripping the sheath off the postganglionic trunk, by keeping the nerve free of excess moisture between the stimulating electrodes and the earthed lead, and by employing an earthed lead as large as possible. An earthed bridge balancing device has not been used.



Text-fig. 1. Diagrams showing the positions of the recording electrodes, *G* (grid) and *E* (earth), and the stimulating electrodes, *P* (preganglionic) and *A* (antidromic). The shaded region shows the extent of the contact of the preganglionic trunk and the ganglion with the body of the cat.

Results

A. *The ganglionic action potential set up by an antidromic volley*

Pl. I, fig. 1, shows typical spike potentials set up by single stimuli applied either to the postganglionic trunk (observations 1 and 3) or to the preganglionic trunk (obervation 2), the arrangement of the stimulating and recording leads being as shown in Text-fig. 1A. Shortly after the stimulus artefact in observation 1, a diphasic action potential begins with a negativity of the lead nearer the stimulating electrodes on the postganglionic trunk. This potential change is obviously due to the volley of impulses propagated antidromically, and from the latent period the approximate conduction velocity may be calculated—in this case about 2 metres a second. There is also a considerably faster volley (at least 4 metres a second), which in observation 1 is largely submerged by the stimulus artefact, for it appears in observation 3 when this artefact is shortened by diminishing the stimulus strength. This volley is transmitted by postganglionic fibres whose threshold and conduction velocity show that they form the S_1 group [Eccles, 1935*a*], the larger and slower volley being mainly in the S_2 group of fibres, though presumably the S_3 and S_4 groups are also included. The absence of this distinct S_1 response with Belgian hares may be correlated with the uniform character of their postganglionic fibres, all groups being non-medullated.

With the upward deflection in observation 1 (Pl. I, fig. 1) the lead on the ganglion becomes negative to the postganglionic lead, thus signalling the arrival of the antidromic volley at the ganglion. The area (potential × time) of this upward deflection is always much larger than the initial downward deflection, suggesting that an antidromic impulse is not blocked at the origin of the axon, but traverses the ganglion cell and possibly the dendrites, setting up there a larger spike response than in the axon. Now a preganglionic volley evokes a response in which with identical leads the initial upward deflection is much larger than the later downward deflection (observation 2), *i.e.* the spike potential evoked from the ganglion is again larger than that from the postganglionic trunk; hence it also appears probable that the impulse discharged from a ganglion cell down its axon traverses in addition that cell and possibly its dendrites [cf. Eccles, 1935*a*, section E]. A comparison of the action potentials shows that the ganglion cell responses evoked by a maximal preganglionic volley are always more asynchronous than those set up by a maximal antidromic volley, and this probably is sufficient to account for the upward deflection in observation 2 being smaller than in observation 1 without assuming that the maximal preganglionic volley fails to set up a discharge from some ganglion cells. This greater asynchronism of the preganglionic response is of course due to the dispersion in the durations of the individual synaptic delays.

In addition to the diphasic spike response an antidromic volley always sets up a slow potential change in which the ganglionic lead is positive to the postganglionic lead (Pl. I, fig. 2, observations 2 and 4). This slow positive wave resembles that set up by a preganglionic volley, being practically confined to the ganglion [cf. Eccles, 1935*c*, section A], and having a similar time course (cf. Pl. I, fig. 2, observations 1 and 3 with 2 and 4), but it always reaches a maximum sooner than the preganglionic slow positivity, and, when both volleys are maximal, it often is considerably smaller in potential. In about half the experiments, however, the maximum positivity is little if any less than that set up by a maximal preganglionic volley, but even then the rate of decay is always quicker for the antidromic response (cf. Text-fig. 3).

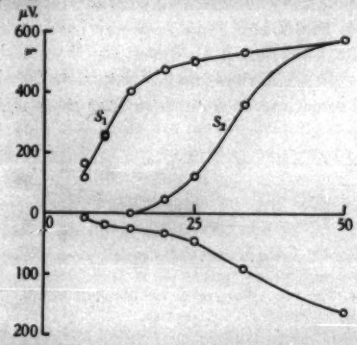
In making this comparison every precaution has been taken to ensure that the antidromic volley is fully maximal. Thus maximality is not assumed until a considerable further increase in the strength of the antidromic stimulus fails to increase either the spike or the slow positivity of the antidromic response. But such strong stimuli may give such large

artefacts that the base-line of the amplifier is displaced throughout the whole duration of the slow positivity. These displacements of the base-line may be detected by a shift of the S_2 diphasic spike relative to the initial base-line, but their later course can only be controlled by two methods to be described later, one depending on the removal of the slow positive wave by nicotine (Pl. I, fig. 4, observation 5), and the other on the occlusion of the positive wave by a P wave set up by a preganglionic volley at various intervals later (Text-figs. 13, 17 B). When the stimulus artefact is thus controlled, an antidromic volley still sets up a slow positive wave smaller than that of a preganglionic volley. As the slow positivity is largely if not entirely produced by the ganglion cells, any injury blocking conduction in postganglionic fibres central to the cathode of the antidromic electrodes would necessarily result in a submaximal antidromic response regardless of the strength of the antidromic stimulus. Doubtless in some experiments such injury has played a part in diminishing the antidromic response, but by careful dissection of the postganglionic trunk this has been avoided as far as possible, and in any case submaximality of the antidromic response would not explain the faster decay of the slow positivity. It may, therefore, be provisionally concluded that a maximal antidromic volley sets up in the ganglion a slow positive wave which occasionally may be almost as large as that set up by a maximal preganglionic volley, but which is often much smaller and always decays more quickly.

Pl. I, fig 3, shows a typical series of antidromic responses elicited by stimuli of different strengths, the absence of an S_2 spike showing that the weakest stimuli (observations 1, 2 and 3) only set up antidromic impulses in S_1 fibres. The small, slow, positive wave set up in these observations must be produced by S_1 ganglion cells. The much larger positive wave in observation 5 must be mostly due to the large S_2 response which is evoked by the stronger stimulus, for the S_1 spike is but little larger than in observation 3. The time course of the S_1 positive wave is slightly quicker than that of the combined response, and hence must be a little quicker than the S_2 positive wave. The relative sizes of the slow positive waves set up by S_1 and S_2 antidromic volleys may be deduced from Text-fig. 2, in which the potentials of the S_1 and S_2 spikes and the slow positive waves are plotted against the respective strengths of the antidromic stimuli. The rapid increase in the P wave is clearly due to the increasing S_2 response. Text-fig. 2 shows that for the same spike potential the slow positive wave of the S_2 ganglion cells is about three times that of

the S_1 ganglion cells. There can be little doubt that this ratio, which is typical of other experiments, is too great to be explained by greater asynchronism of the S_2 response giving rise to a relatively greater reduction in the S_2 spike; hence it seems likely that an antidromic impulse evokes a slow positive wave whose potential relative to the spike potential is greater for an S_2 than for an S_1 ganglion cell.

Analysis by varying the strength of the preganglionic stimulus, by the action of nicotine in various concentrations, and by interaction of successive preganglionic volleys, has shown that the slow potential wave

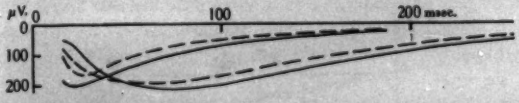


Text-fig. 2. The potentials of the S_1 and S_2 antidromic spikes are plotted as ordinates against as abscissæ the respective strengths of the stimuli in arbitrary units. The potentials of the corresponding slow positive waves are plotted downwards.

set up in the ganglion by a preganglionic volley is composed of a negative wave *N* overlapping a slower positive wave *P* [Eccles, 1935*c*], both waves reaching their maximum at about 20 msec., but *N* decaying at more than twice the rate of *P*. The more rapid decay of *N* results in the phase of increasing positivity for 100–150 msec., and beyond this point of maximum positivity the absolute rate of decay of *N* is less than that of *P*. Pl. I, figs. 2, 3 and 4, show that the slow potential wave set up by an antidromic volley also has a phase of increasing positivity, though it usually does not last for more than 100 msec., and this suggests that this slow positive wave is also compounded of a short negative wave *N* running concurrently with a positive wave *P*. Moreover, painting the ganglion with dilute nicotine always removes simultaneously the phases of increasing positivity with both preganglionic and antidromic responses

(Pl. I, fig. 4, observations 3, 4). The provisional conclusion that an antidromic volley sets up an N wave similar to that set up by a preganglionic volley is confirmed by experiments on the interaction of antidromic and preganglionic volleys in section C.

After the removal of the N wave by nicotine the slow positive wave is seen to be maximal right at the end of the ganglionic spike, hence it may be concluded that normally the P wave is maximal not later than this point, i.e. within 20 msec. of the setting up of the antidromic volley. The N wave also must normally reach the maximum within 20 msec., and so both the N and P waves set up by an antidromic volley have time courses similar to the N and P waves set up by a preganglionic volley [cf. Eccles, 1935*c*, Text-fig. 9]. However, the N wave set up by a maximal antidromic volley is always smaller than that set up by a preganglionic volley and sometimes it is much smaller (Pl. I, fig. 2), for with an



Text-fig. 3. Superimposed curves of the slow potential waves shown in Pl. I, fig. 4, the preganglionic responses being shown by the continuous lines and the antidromic by the broken lines. The two lower curves are obtained before, the two upper after painting the ganglion with 0·005 p.c. nicotine.

antidromic volley the N wave is always smaller relative to the P wave than it is with a preganglionic volley. In some experiments this relationship is obscured, for the early part of the antidromic N wave always suffers an apparent increment on account of the overlapping latter part of the antidromic spike response of the ganglion, which is of course in the same direction; while, conversely, the early part of the preganglionic N wave always appears diminished on account of the diphasic artefact of the spike response. The smaller antidromic N wave accounts for the earlier point of maximum positivity after an antidromic volley, and the allowance for the larger preganglionic N wave would also result in the preganglionic P wave being larger relative to the antidromic P wave than at first appeared from comparison of the slow positive waves.

Pl. I, fig. 4, observations 3 and 4, shows that, in addition to removing the N wave, nicotine also shortens and diminishes the P waves set up both antidromically and preganglionically [cf. Eccles, 1935*c*, section C]. However, it will be seen in Text-fig. 3 that the preganglionic P wave is

still larger. This identical sensitivity to nicotine suggests that an antidromic volley sets up *N* and *P* waves exactly like those set up preganglionically.

In Pl. I, fig. 4, observation 5, a still larger dose of nicotine has almost abolished all the slow positive wave after the spike. It is possible that the small remaining positive wave is due to displacement of the base-line by the stimulus artefact. In this way the action of nicotine provides a control demonstrating that the stimulus artefact is certainly reduced to negligible proportions within 50 msec. This dose of nicotine, while hardly affecting the antidromic spike response, almost completely abolishes the spike set up by a preganglionic volley (observation 6). It may, therefore, be concluded that nicotine prevents a preganglionic volley from setting up a discharge of an impulse from a ganglion cell by a block central to the point to which an antidromic impulse can penetrate.

When both amplifier leads are on the preganglionic trunk, an antidromic volley does not give rise to any action potential, thus confirming the irreversibility of transmission through the ganglion observed by Bishop & Heinbecker [1932] and Brown [1934]. Moreover, when one amplifier lead is on the ganglion and one on the preganglionc trunk, only a very small action potential is produced by an antidromic volley, the preganglionic trunk acting as a non-specific lead from the ganglion cells and the postganglionic fibres [cf. Eccles, 1935*a*]. With concentric needle electrodes in the ganglion an antidromic volley gives rise to a diphasic spike potential, the first phase being as usual of opposite sign to that set up by a preganglionic volley. The slow potential waves produced by the antidromic volley were not originally detected with these leads [Eccles, 1934], for they were obscured by the large slow stimulus artefact.

Discussion. When an antidromic volley is backfired into motoneurones of the frog's spinal cord, Umrath [1933] recorded a series of potential changes very similar to those described here for ganglion cells—an initial spike followed by slow negative and positive waves—but he suggested that the slow negative wave was the action potential produced by the antidromic impulses traversing the motoneurones. Stimulation of the dorsal roots produced the same sequence of waves, both being larger and the positive wave longer, but these waves presumably would be partly produced by internuncial neurones. Application of 1 p.c. phenol to the spinal cord removed the negative wave set up by a dorsal root volley, thus resembling dilute nicotine action on the ganglion, but contrary to the ganglion observations an antidromic volley still produced its full *N* wave.

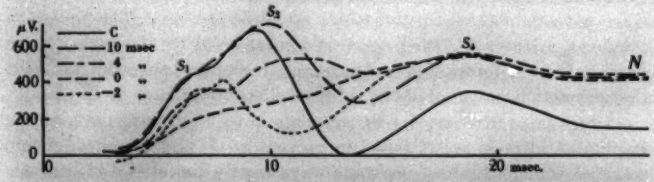
In apparent contradiction to Umrath, Gasser & Graham [1933] and Hughes & Gasser [1934*a*] found that an antidromic volley set up a spike action potential but no slow waves in the cat's spinal cord. However, the leads employed by Gasser and his co-workers are unsuitable for detecting potential waves developed by the motoneurones, and, on account of Umrath's observations on the frog, it seems likely that such waves would be recorded with suitable leads, for as shown by Gasser and his co-workers and Barron & Matthews [1936] nerve cells in the spinal cord certainly develop slow *N* and *P* waves, and one would expect frog's motoneurones to resemble a cat's motoneurones at least as closely as a cat's ganglion cells. The spike action potential followed by *N* and *P* waves might, therefore, well be the characteristic response evoked in any nerve cell by an antidromic impulse.

B. *The refractory period set up by an antidromic volley*

Pl. II, fig. 5, shows a typical series of ganglionic spike potentials set up when single maximal antidromic and preganglionic volleys are fired into the ganglion at various short intervals apart, the control observations 1 and 6 being respectively the isolated preganglionic and antidromic responses. In observation 5 a preganglionic volley clearly gives rise to a spike potential resembling the control but a little smaller than it, showing that at this interval (11·4 msec.) the ganglion is but little affected by the preceding antidromic volley; while in observation 4 an antidromic stimulus applied 11·9 msec. after the preganglionic produces almost no change in the subsequent course of the preganglionic action potential, indicating that at the time of the antidromic stimulus most of the postganglionic fibres are refractory—presumably on account of the volley just previously discharged along them in response to the preganglionic volley.

The other observations of Pl. II, fig. 5, require analysis. Now if the antidromic volley is not too late relative to the preganglionic volley, it will excite all the ganglion cells before any impulses have been set up in them by the preganglionic volley. Under such conditions it may be assumed that the spike potential of the antidromic volley is unaffected by the preganglionic volley; hence the action potential set up by the latter volley can be determined by subtracting the antidromic action potential from the combined action potential. The subtracted action potentials of Text-fig. 4 show, however, a complication that is always introduced by the slow potential waves. Thus a preganglionic volley 10 msec. after an antidromic volley sets up an S_2 spike which in the subtracted curve is actually

higher than the control, but it declines much less, for there is apparently a much larger slow negative wave. It will be seen later (section C(3)) that this is really due to the occlusion by the antidromic *P* wave of most of the *P* wave set up by the preganglionic volley. The *N* wave of the subtracted curve therefore resembles the preganglionic *N* wave as it would appear in the presence of a much smaller *P* wave. If allowance be made for the *P* wave occlusion, the potential of the S_2 spike of the subtracted curve is less than that of the control response, and the summit is about 0·6 msec. later. When the preganglionic volley follows the antidromic at a shorter interval (4 msec., Text-fig. 4), the S_2 spike is still smaller and later, and the S_1 spike is also smaller and perhaps a little delayed. Finally, when the preganglionic and antidromic stimuli are



Text-fig. 4. The continuous curve shows the ganglionic potential set up by a maxima preganglionic volley, the other curves being the subtracted action potentials for this volley when it is set up at the indicated intervals later than a maximal antidromic volley. At the interval indicated as −2 msec. the preganglionic volley preceded the antidromic volley by 2 msec.

simultaneous, there is no sign of either an S_1 or an S_2 spike in the subtracted curve, which ascends gradually for about 20 msec., being probably an *N* wave [cf. Eccles, 1935*c*, Text-figs. 6*c*, 9] complicated only by the unoccluded part of the *P* wave and the late S_4 spike indicated in the figure. If allowance be made for synaptic delays and the pre- and post-ganglionic conduction times, this curve shows that firing an antidromic volley into the ganglion prevents a preganglionic volley from setting up a discharge of the S_1 and S_2 ganglion cells for at least 2·5 and 5 msec. respectively. The curve at a stimulus interval of 4 msec. shows that recovery of S_1 and S_2 ganglion cells is well advanced 6·5 and 9 msec. respectively after an antidromic volley, while the curve at 10 msec. interval shows that 12·5 msec. after an antidromic volley the S_1 recovery is almost complete, though the S_2 response is still considerably diminished and delayed. It will be seen in section D that especially with S_2 ganglion cells the refractory period overlaps with a later period of depression.

Text-fig. 4 (interval −2 msec.) illustrates a change in the subtracted curve that always arises if the preganglionic volley is placed very early relative to the antidromic volley. The subtracted curve shows an early spike and it is followed by a trough, i.e. it resembles a diphasic spike. This apparent diphasic response is even more obvious when the preganglionic volley is moved yet farther forward. It clearly cannot be a preganglionic response subsequent to the antidromic volley, for even when earlier, e.g. at 0 interval, this volley blocks all response to a preganglionic volley. Now the subtraction method for determining the preganglionic response is no longer applicable when the antidromic volley fails to excite some ganglion cells before impulses have been set up in them by the preganglionic volley; for, as no spike potential would be produced in these cells by the antidromic volley, the control antidromic spike potential (which is subtracted) would be too large. The trough so produced in the subtracted curve is compensated by the earlier spike potential of the forestalling preganglionic response, hence the apparent diphasic action potential of the subtracted curves at such intervals.

Altogether thirteen other experiments have been performed, but in ten the antidromic volley failed to prevent an S_1 and S_2 preganglionic response, though this response was always greatly diminished at intervals such as at 0 and 4 msec. of Text-fig. 4. Presumably in such experiments the antidromic volley was not maximal, and this may be attributed either to the stimulus being too weak or to damage to the postganglionic trunk.

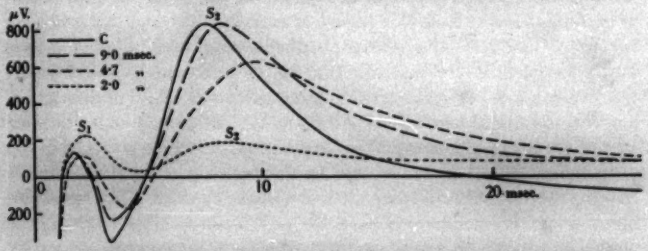
Brown [1934] similarly found that, in some experiments on S_1 ganglion cells in the superior cervical ganglion, an antidromic volley (presumably maximal) failed at all intervals to interfere completely with the discharge of impulses set up from the ganglion cells by a preganglionic volley. Rejecting as unlikely a repetitive ganglion cell discharge in response to a single preganglionic volley, he suggested that an asynchronism of more than 2 msec. in this discharge would be sufficient to account for the incomplete interference; but, as will be seen in a later paper, his observations show that an asynchronism of at least 5 msec. is necessary. Usually, however, there was an absolute and later a relative interference (as in Text-fig. 4) between the antidromic volley and the discharge set up by the preganglionic volley, and the experiments where this cannot be demonstrated presumably are explicable without being exceptions to the general statement that an antidromic impulse sets up in the ganglion cell a short period of depression during which a preganglionic volley cannot excite it to discharge an

impulse. No precise measurement of this period is possible in our experiments, and, as Brown's experiments give no indication of the time of the earliest discharge set up after an antidromic volley, no figures are deducible from them; however, the above experiments provide no reason for supposing that this period differs in duration from the period of similar inexcitability determined by two preganglionic volleys, for which a value of 3 msec. is probably the best estimate for S_1 ganglion cells [Eccles, 1935*a*, section I]. This period may be termed a functional refractory period [cf. Derbyshire & Davis, 1935]. It must be longer than the true absolutely refractory period, for the testing preganglionic volley provides a stimulus to the ganglion cells of only a limited strength.

Brown [1934] gives a value of 15 msec. for the duration of the period of gradual recovery from this period of inexcitability, and this is in good agreement with out experiments, e.g. the recovery of S_1 ganglion cells is nearly complete in 12·5 msec. in Text-fig. 4. It is most improbable that this depression is caused by an absolutely refractory period of some ganglion cells, i.e. an absolute block set up by the antidromic volley in some part of the postganglionic-ganglionic pathway, for within a similar group of ganglion cells there would not be the wide range of absolutely refractory periods from 3 msec. to more than 15 msec. The depression must, therefore, be due to the relatively refractory period following the antidromic volley [cf. Eccles, 1931, p. 565]. The preganglionic volley excites some ganglion cells so strongly that they discharge impulses very early in this relatively refractory period, e.g. after 3 msec., others are excited only just above their normal threshold and so can only discharge impulses at the very end of the relatively refractory period, e.g. after 15 msec., and all transitions exist. Since, therefore, this relatively refractory period set up by an antidromic volley raises the threshold at which a ganglion cell responds to excitation by preganglionic impulses, the antidromic volley must penetrate at least as far as the locus at which the ganglion cell discharge is set up; hence distal to this locus conduction is reversible.

A closer approximation to the true absolutely refractory period of the ganglion cells may be determined by analysing by the subtraction method the ganglionic action potentials produced by two antidromic volleys at various intervals apart. Text-fig. 5, which is typical of such experiments, shows that, when the stimulus interval is as short as 2 msec., the second volley produces both an S_1 and a small S_2 spike response, but after a delay longer than the normal control response by about 0·5 msec. for S_1 and 1·0 msec. for S_2 ganglion cells. At a stimulus interval of 4·7 msec. the

S_2 spike begins earlier, but its summit is further delayed, presumably on account of the slower postganglionic fibres and their ganglion cells which were not stimulated at 2 msec. interval. Finally at 9 msec. interval the S_1 spike and the beginning of the S_2 spike are almost normal, but the summit of the S_2 spike is delayed by about 0·7 msec., and its potential is lower if allowance be made for the occlusion of the P wave (cf. section C (1)), which is indicated by the subtracted curves declining less than the control curve (cf. Text-fig. 7). It may therefore be concluded that, as far as the antidromic impulse penetrates, there is almost complete recovery from the relatively refractory period of the S_1 ganglion cells at 9 msec., and recovery of the S_2 ganglion cells is then far advanced.



Text-fig. 5. The continuous curve shows the ganglionic action potential evoked by a maximal antidromic stimulus, the other curves being the subtracted action potentials produced by it when it is preceded at the indicated intervals by an antidromic volley set up by a similar maximal antidromic stimulus.

Now even at stimulus intervals as short as 4·7 and 2 msec. the subtracted S_2 spike appears to have its normal diphasic character, indicating that at such intervals, if the second stimulus sets up an antidromic impulse in a postganglionic fibre, that impulse penetrates as far as normally into the ganglion cell; hence it may be concluded that the absolutely refractory period of a ganglion cell is little if any longer than that of its axon, a conclusion similar to that of Lorente de Nó [1935*b*] for the motoneurones of the internal rectus muscle. The increased delay of the ganglionic spikes set up by the antidromic stimuli in Text-fig. 5 is presumably due to the slower conduction of the antidromic volley during the relatively refractory period of the postganglionic fibres. The absolutely refractory period of the ganglion cells is determined by adding this extra delay to the shortest stimulus interval at which a response was evoked, 2 and 3 msec. being the upper

limiting values so determined for the refractory periods of the fastest S_1 and S_2 ganglion cells.

The delay of the S_2 spike summit and diphasic artefact in Text-fig. 4 has been characteristic of all our experiments, and though in many it has been greater than in Text-fig. 4, a delay of more than 2·5 msec. has never been observed. A selective blocking of the discharge from those ganglion cells which normally have the shortest synaptic delays would perhaps be an adequate explanation of the small delay of the S_2 spike in some experiments, but delays as long as or longer than those of Text-fig. 4 certainly indicate that there is a lengthening of the synaptic delay associated with the relatively refractory period set up by the antidromic volley. Slowed conduction in the postganglionic pathway cannot of course be invoked as an explanation, for the delayed spike is recorded directly from the ganglion cells. As the preganglionic volley is set up progressively earlier relative to the antidromic volley, the synaptic delay appears in each experiment to reach a limiting value beyond which it cannot be lengthened, for any further displacement of the preganglionic volley diminishes the S_2 discharge to extinction without further altering its synaptic delay. The conditions obtaining for S_1 discharge appear to be similar, but the lengthenings are smaller, and cannot be so easily demonstrated in records of action potentials on account of the overlapping S_2 wave. This question will be further considered in a later paper.

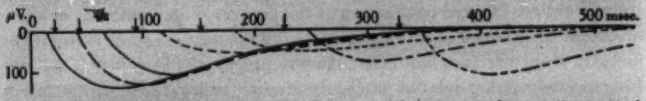
When a refractory period of ganglion cells was set up by a preganglionic volley, the synaptic delay of S_1 and S_2 ganglion cells was also found to be increased at very short intervals [Eccles, 1935*a*, section I], but the simultaneous facilitating influence of the initial preganglionic volley rapidly overcame this effect of the refractory period. A lengthening of the synaptic delay of the spinal flexor reflex is also produced by the refractory period set up in motoneurones by an antidromic volley [Eccles, 1931], but Lorente de Nó [1935 *b*] states that an antidromic volley produces if anything a shortening of the synaptic delay of the internal rectus motoneurones. However, in some at least of the records which are referred to in support of this statement, antidromic impulses would be prevented from reaching some motoneurones by impulses discharged from these motoneurones. Moreover, his conclusions depend on the interpretation of muscle action potentials, i.e. of responses beyond the neuromuscular junction, and so will be complicated by the increased neuromuscular delay of the second response during the relatively refractory period, a curve of Lucas' type C actually being obtained for the nerve muscle preparation [Lorente de Nó, 1935 *a*]. The effect of anti-

dromic impulses on motoneurones of the third nerve may not therefore be different from their effect either on motoneurones of the spinal cord or on ganglion cells.

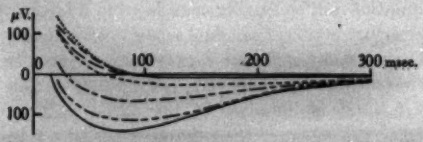
C. *Interaction of the slow potential waves set up in the ganglion by two successive volleys*

(1) *Two antidromic volleys.*

If, during the phase of slow positivity produced in the ganglion by an antidromic volley, a second antidromic volley be set up, there is always a brief diminution of this positivity [cf. Eccles, 1935 *c*, section F,



Text-fig. 6. The continuous line shows the slow potential wave set up by a single maximal antidromic volley (the initial spike not being drawn in this and all subsequent figures), and the other curves (cf. Pl. II, fig. 6) show the course of the slow potential waves after a second maximal antidromic volley for the stimulus intervals indicated by the arrows (the corresponding curves beginning about 20 msec. later). The latter part of the curve at a stimulus interval of 43 msec. (dotted line) is not drawn as it practically coincides with that at 21·5 msec.



Text-fig. 7. The subtracted slow potential waves set up by the second antidromic volley for each of the corresponding combined responses shown in Text-fig. 6, the same conventions of line being used. The latter parts of the curves for the three shortest stimulus intervals coincide in the continuous line just below the zero line.

interaction of preganglionic volleys], followed by its redevelopment (Pl. II, fig. 6). If, however, the observations at the various intervals are superimposed with the first volleys synchronized (Text-fig. 6), the slow positivity following the second antidromic volley is found, for stimulus intervals less than 100 msec., to be very little greater than that set up by the first volley alone, i.e. the second volley has produced very little change in the course of the slow positivity. As the stimulus interval is lengthened beyond 100 msec., the second antidromic volley gradually recovers its power to produce its normal full-sized slow positivity, but

there is with stimulus intervals of about 150 msec. a well-developed minimum for the combined slow positivity, Text-fig. 6 being typical of all such experiments (six in all).

The closeness with which at short stimulus intervals the combined slow positivity follows the control slow positivity suggests that the slow positivity set up by the first antidromic volley continues its course unaffected by the second volley, which, however, itself produces little additional slow positivity. This provides a method for determining the course of the slow potential set up by the second volley, the slow potential of the first volley being subtracted from the combined slow potential [cf. Hughes & Gasser, 1934 *b*; Eccles, 1935 *c*, section E]. Subsequent evidence justifies this procedure, but a conclusive proof of its correctness is not at present possible. The subtracted curves so determined (Text-fig. 7) show that the second antidromic volley produces an increased slow negativity at the same time as the decreased slow positivity.

On the basis of the analysis of the slow potentials into *N* and *P* waves (section A) the decreased slow positivity is clearly due to a diminished *P* wave, i.e. there seems to be an occlusion of the second *P* wave by the pre-existent *P* wave, which, however, must be a delayed action, for Text-fig. 6 shows that the occlusion does not diminish until the pre-existent *P* wave is considerably reduced; hence the well-developed minimum of slow positivity in the combined response. Likewise the increased slow negativity appears to be largely due to a diminution of *P*, and not to a great increase in *N*. However, the occurrence of a small change in *N*, which would bring it into line with the observations on preganglionic volleys [Eccles, 1935 *c*, section F], is typically shown by the observations of Text-fig. 7 at the short intervals. While the stimulus interval lengthens from 21·5 to 94 msec., and the slow positivity (and hence the *P* wave) remains unaltered, the slow negativity (and hence the *N* wave) first increases and then diminishes. The initial increase is much more obvious if still shorter intervals are chosen, and it shows that, when set up during the *N* wave of the first antidromic volley, the second antidromic volley produces a diminished *N* wave, i.e. there is an occlusion of this *N* wave. Further, the increase of the *N* wave to a maximum, which occurs when the second antidromic volley is set up during the phase of the greatest positivity following the first antidromic response, indicates that the *N* wave is increased when it is set up during a background *P* wave.

If the increase in the slow negativity of the second response be plotted against the stimulus interval, a curve is obtained closely resembling an inversion of the slow potential wave of the first response (cf. Text-figs. 13

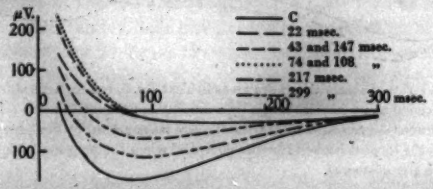
and 14 for a second preganglionic volley). This agreement suggests that the pre-existent N and P waves are directly responsible for the changes in the N and P waves set up by the later antidromic volley (cf. section C(3)). Thus qualitatively the interaction of the N and P waves set up by two antidromic volleys is similar to that observed for the interaction of two preganglionic volleys, the only differences being the much larger occlusion of the antidromic P waves, and the smaller increase of the antidromic N wave when set up in a background P wave.

(2) *Interaction of a preganglionic with a later antidromic volley.*

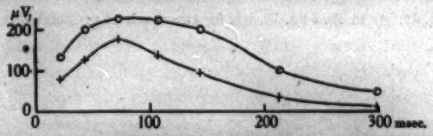
When an antidromic volley is set up during the slow potential wave following a preganglionic volley, the effect on its slow potential wave resembles that produced by a preceding antidromic volley (Pl. II, fig. 7). However, when the observations at various intervals are plotted with the initial preganglionic volleys superimposed, the antidromic volley usually produces a greater augmentation of the slow positivity than in Text-fig. 6 (the two exceptional experiments are not reliable, as they are complicated by a large artefact produced by the antidromic stimulus). The subtracted curves in Text-fig. 8 differ from those of Text-fig. 7 only in the larger P wave with short stimulus intervals, and the same conclusions as in the previous section may be drawn with regard to N and P wave interaction, thus further supporting the evidence of section A in proving the identity of the N and P waves set up antidromically and preganglionically.

If the heights of the S_2 ganglionic spikes of the second antidromic responses be measured from the base-lines provided by the previous responses (cf. Pl. II, figs. 6 and 7), these heights are found to be increased relative to the normal control whether the preceding volley be antidromic or preganglionic. When plotted against the stimulus interval, the increase of the spike height always runs approximately parallel to, but at a lower level than, the increase in the slow negativity measured immediately after the spike (Text-fig. 9). It, therefore, seems probable that the increased spike height is an indicator of the partial completion of the increased negativity produced by the occlusion of the P wave and, presumably, the increase of the N wave, these effects being thus shown to be more than half completed at the time of the S_2 ganglionic spike, which in Text-fig. 9 was only 6·7 msec. after the antidromic stimulus. Since probably 3 msec. would be occupied in the conduction time of the S_2 antidromic impulses, the N and P waves must normally be set up almost immediately these impulses reach the ganglion cells, and the waves must

quickly reach a maximum, for this was certainly attained in the upper curve of Text-fig. 9 only 18 msec. after the antidromic stimulus. Such a rapid increase of the *N* and *P* waves is also indicated by the experiments in which nicotine removed the *N* wave, for the *P* wave appeared to be maximal at least as soon as the end of the antidromic spike (Pl. I, fig. 4), and it is in agreement with the rapid increase also found for the *N* and *P* waves set up by a preganglionic volley (section B) [cf. Eccles, 1935 *c*, sections C and F].



Text-fig. 8. Subtracted slow potential waves as in Text-fig. 7, but for a maximal antidromic volley preceded at the indicated intervals by a maximal preganglionic volley (observations shown in Pl. II, fig. 7). The continuous line is the response to the antidromic volley alone. The curves for stimulus intervals of 43 and 147 msec. practically coincide throughout their course, as also do those at 74 and 108 msec.

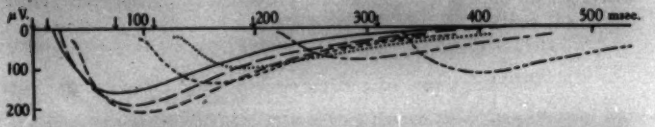


Text-fig. 9. For the crosses the increase in the antidromic S_1 spike is plotted against the stimulus interval, and for the circles the increase in the slow negativity immediately after the spike is similarly plotted, the observations being partly shown in Pl. II, fig. 7.

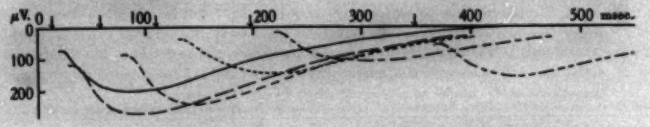
(3) *Interaction of an antidromic with a later preganglionic volley.*

Pl. III, fig. 8, shows that a maximal preganglionic volley always adds considerably to the slow positivity set up by a maximal antidromic volley, the interaction thus differing from that occurring between two antidromic volleys. When the curves are plotted with the antidromic volleys synchronized (Text-fig. 10), this addition to the slow positivity is well seen, and the close similarity to the interaction between two preganglionic volleys is revealed by Text-fig. 11, where such observations from the same experiment are similarly plotted. Despite the considerable addition to the slow positivity, this in Text-figs. 10 and 11 passes through well-

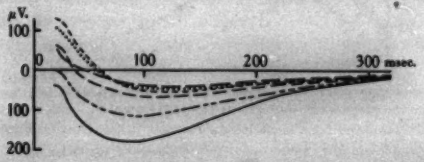
developed minima with stimulus intervals of about 200 msec. (cf. Text-fig. 6). The smaller positivity with simultaneous stimuli in Text-fig. 10 is observed in all experiments when the stimulus intervals are so short that the refractory period of the antidromic volley prevents the preganglionic volley from setting up a discharge from some or all of the ganglion cells (cf. section B).



Text-fig. 10. A series of observations drawn as in Text-fig. 6, but for a maximal preganglionic volley preceded at various intervals by a maximal antidromic volley (cf. Pl. III, fig. 8). The continuous curve shows the slow potential wave set up by the preganglionic volley alone, and the longest broken line the potential when the preganglionic and antidromic volleys are simultaneous.



Text-fig. 11. As in Text-fig. 10, but for two maximal preganglionic volleys.



Text-fig. 12. Subtracted curves as in Text-figs. 7 and 8, but for the series of observations shown in Text-fig. 10, the line conventions again corresponding. The curve for the interval of 199 msec. was not plotted as it was practically superimposed on that at 19 msec.

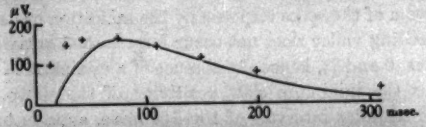
Besides this minimum at simultaneity the subtracted preganglionic potentials, i.e. the ganglionic potentials set up by the preganglionic volley, also usually show a minimum slow positivity with intervals of about 100–150 msec. (cf. dotted curve, Text-fig. 12), which has never been observed with subtracted antidromic potentials (cf. Text-figs. 7 and 8). This minimum is sometimes also present with the interaction of two preganglionic volleys (cf. curve at 109 msec. interval, Text-fig. 11), but

has not been detected when the inhibition of the second preganglionic volley is small (cf. Eccles, 1935 *c*, Text-fig. 12). Moreover, this minimum occurs over just that range of intervals at which the inhibition is maximal, hence it is concluded that it results from the diminution of the second preganglionic discharge by the inhibitory effect of the first volley. The diminution of the spike response to a preganglionic volley, which is also produced by a preceding antidromic volley (see section D and Pl. III, figs. 8 and 10), similarly would account for the minimum of Text-fig. 12. This diminution of the spike response by the inhibition or depression set up by a preceding volley does not occur for a second antidromic volley (cf. Pl. II, figs. 6 and 7), hence the absence of a minimum in Text-figs. 7 and 8. Thus the minimum slow positivity of the subtracted action potentials at stimulus intervals of 100–150 msec. appears to be related to that at very short stimulus intervals, both being due to the failure of the preganglionic volley to set up a discharge from some ganglion cells.

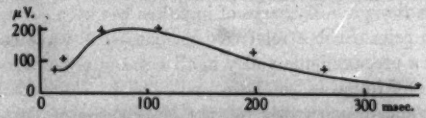
When the preganglionic volley is simultaneous with the antidromic volley, the subtracted curve in Text-fig. 12 shows that it produces a large *N* wave even though a discharge of impulses has been set up from very few ganglion cells. Such a relatively unchanged *N* wave has also been produced by a preganglionic volley in all series of observations in which the discharge of impulses has been prevented by the refractory period following an antidromic volley (cf. the observation of Text-fig. 4 with simultaneous stimuli). The production of the *N* wave by a preganglionic volley is therefore but little affected by a refractoriness of the ganglion cells which prevents the discharge of impulses, thus differing from the production of the *P* wave which is considerably diminished though apparently not abolished under such conditions.

In Text-fig. 13, for the series of observations of Text-fig. 12, the maximum increase of the preganglionic slow negativity has been plotted against the stimulus interval. Except for the shortest stimulus intervals the plotted points lie very close to the curve in Text-fig. 13, which shows the inverted course of the slow potential set up by the initial antidromic volley. There is a similar good agreement in all such series of observations when the artefact produced by the antidromic stimulus is small, and also, as shown in Text-fig. 14, in series obtained by the interaction of two preganglionic volleys. This agreement provides additional evidence supporting the conclusion that the pre-existent *N* and *P* waves are directly responsible for the modifications in the *N* and *P* waves set up by a preganglionic volley [cf. Eccles, 1935 *c*, section F; and section C(1) where the *N* and *P* waves set up by an antidromic volley are similarly

affected]. The maximum increase in the slow negativity set up by a preganglionic volley at various times after an antidromic or preganglionic volley may therefore be used to construct the approximate course of the slow potential set up by these volleys, a construction which is useful in comparing the slow potentials set up by antidromic and preganglionic volleys when the antidromic potential is distorted by stimulus artefact (cf. Text-fig. 17 B).



Text-fig. 13. The points show the stimulus intervals plotted against the corresponding maximum increases of the slow negativity set up by a preganglionic volley (series partly shown in Text-figs. 10 and 12). The curve shows the inverted course of the slow potential wave set up by the antidromic volley (cf. continuous line in Text-fig. 10).



Text-fig. 14. As in Text-fig. 13, but for the two maximum preganglionic volleys of Text-fig. 11. The curve shows the inverted course of the slow potential wave set up by the preganglionic volley.

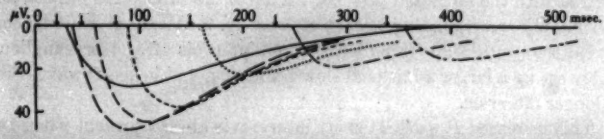
The minimum of the subtracted slow positivity at intervals of about 120 msec., and the larger fraction of its slow positivity which is added by a preganglionic volley, provide the only points in which the interaction of an antidromic with a preganglionic volley differs from the interactions considered in the preceding sections. It may therefore be concluded that in all the possible interactions between antidromic and preganglionic volleys a pre-existent *P* wave in excess of *N* diminishes the *P* wave and increases the *N* wave set up by a later volley, while a pre-existent *N* wave similarly diminishes the *N* wave set up [cf. Eccles, 1935 *c*].

(4) *Interaction of submaximal volleys.*

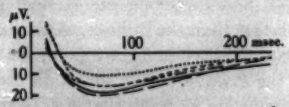
When testing the interaction between a submaximal preganglionic and a submaximal antidromic volley, it must be recognized that there will only be a partial overlap of the fields of ganglion cells acted on by

these respective volleys. However, when testing interaction between two preganglionic volleys or between two antidromic volleys which are set up by identical stimuli applied through the same electrodes, the incompleteness of the overlap probably does not reach significant proportions, for the spontaneous changes of excitability [Blair & Erlanger, 1933, 1936] are probably very small.

The interaction of two submaximal antidromic volleys exhibits a behaviour identical with that described for maximal volleys, and this is observed even when both volleys are restricted to S_1 fibres; hence it may be concluded that with antidromic volleys the interaction of N and P waves is similar for S_1 and S_2 ganglion cells.



Text-fig. 15. A series of observations plotted as in Text-fig. 6, but for a submaximal preganglionic volley followed by a submaximal antidromic volley (cf. Pl. III, fig. 9).



Text-fig. 16. Subtracted potentials as in Text-fig. 8, but for the submaximal responses of Text-fig. 16, a similar line convention being used, but in order to avoid confusion only some observations are plotted.

In investigating the interaction between submaximal antidromic and preganglionic volleys a relatively large overlap may be ensured by employing stimuli which are almost maximal for the respective preganglionic and postganglionic S_1 fibres, for such stimuli are then almost below threshold for the S_2 fibres, and there is an almost complete overlap on the S_1 ganglion cells.

The interaction of two such volleys is illustrated in Pl. III, fig. 9, and in Text-fig. 15 the initial preganglionic volleys are synchronized for the various responses. With short stimulus intervals, e.g. observations 4 and 7, the large additional slow positive wave distinguishes these observations from those with maximal stimuli (Text-fig. 8). The subtracted curves (Text-fig. 16) show that the added positive wave may even be larger

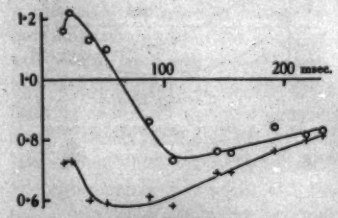
(interval 21 msec.) than the positive wave set up by the antidromic volley alone. As the stimulus interval lengthens, the positive wave of the subtracted curve decreases to a minimum of about half the maximum value (interval 148 msec.), increasing again with very long intervals. This decrease to a minimum proves that with short stimulus intervals the large positivity of the subtracted curves is not due to an absence of overlap between the fields of ganglion cells acted on by the preganglionic and antidromic volleys. The unlikely explanation of the minimum by an inhibition or depression of ganglion cells blocking the entrance of antidromic impulses (cf. for maximum preganglionic volley, section C (2)) is contraindicated by the ganglion spike potentials (cf. Pl. III, fig. 9, observation 3), which show on the contrary a small increase running parallel with the increased slow negativity of the subtracted curves, and presumably corresponding to that of Text-fig. 9. Hence it must be concluded that antidromic impulses at short intervals after a preganglionic volley set up a larger additional slow positivity, and hence *P* wave, than at longer intervals.

This increased *P* wave at short intervals is always present when two weak preganglionic volleys interact [Eccles, 1935*c*, section H], the experimental evidence suggesting that the production of *P* wave by preganglionic impulses is increased by a pre-existent slow negativity, i.e. when the *N* wave is larger than the *P* wave. A similar explanation seems likely for the increased *P* wave in Text-fig. 16, for the *N* wave is there initially in excess of the *P* wave, such a condition always obtaining when the preganglionic stimuli are so weak that practically only the S_1 ganglion cells are excited; hence it may be concluded that a pre-existent *N* wave in excess of *P* increases the production of *P* wave by an antidromic as well as by a preganglionic volley. With a maximal preganglionic volley the large *P* wave of the S_2 ganglion cells is usually greater than the *N* wave, there being no phase of slow negativity, hence even at short intervals there is a large occlusion of the antidromic *P* wave (Text-fig. 8). Presumably the antidromic volley would still set up the facilitated *P* wave in the S_1 ganglion cells, but this would form a hardly detectable fraction of the combined S_1 and S_2 responses to maximal volleys.

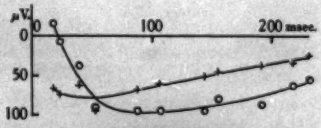
D. *Actions of an antidromic volley on the excitability of ganglion cells*

Pl. III, fig. 10, shows part of a series of observations in which the excitability of S_2 ganglion cells is tested by their response to a submaximal preganglionic volley at various intervals after either a maximal

antidromic volley or a maximal preganglionic volley, and in Text-fig. 17 A the potential of the testing preganglionic spike response is plotted against the stimulus interval for the whole series of observations. The curve thus obtained after an initial preganglionic volley is of course the inhibitory curve of Text-figs. 11, 12 and 15 in a previous paper [Eccles, 1935 *b*], resembling the two former in having a preponderating facilitatory effect at short intervals. On the other hand, an antidromic volley is followed by a large depression of the excitability of the S_2 ganglion cells even at the shortest interval, this depression overlapping with the brief



Text-fig. 17 A.



Text-fig. 17 B.

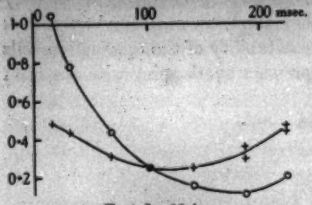
Text-fig. 17 A. The potentials of the S_2 spike responses evoked by the testing preganglionic volley (measured from the summit to the diphasic artefact and expressed as a fraction of the normal control responses similarly measured) are plotted against the stimulus intervals after either a maximal antidromic volley (crosses) or a maximal preganglionic volley (circles), part of the series being shown in Pl. III, fig. 10.

Text-fig. 17 B. The maximum increase in the slow negativity of the testing preganglionic response is plotted downwards against the stimulus interval after a maximal antidromic volley (crosses) or a maximal preganglionic volley (circles). The same series of observations as in Text-fig. 17 A.

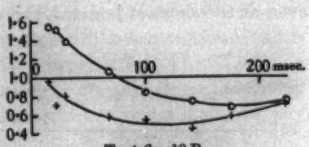
depression regarded as characteristic of the relatively refractory period (section B). After a brief initial increase the depression soon diminishes and at the longest intervals is very little more than after a preganglionic volley. This relationship between the two curves is characteristic of nine of our experiments. The other six experiments have differed only in the larger depression produced by the preganglionic volley at long intervals (cf. Text-fig. 18 A).

An antidromic volley sets up a depression of excitability of S_1 ganglion cells which even at long intervals is never less than any depression following a preganglionic volley (Text-fig. 18 B). However, as shown in Text-fig. 19 a preganglionic volley producing a large facilitatory effect may not give rise to any late depression of excitability, though an

antidromic volley produces its normal depression. At short intervals after an antidromic volley there may actually be a period of facilitation of S_1 ganglion cells separating the brief depression of the relatively refractory period (section B) from the later prolonged depression. In the experiment illustrated by Text-fig. 18 B there would be no continuity between these



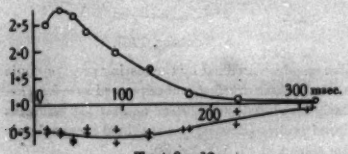
Text-fig. 18 A.



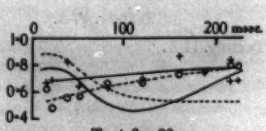
Text-fig. 18 B.

Text-fig. 18 A. For S_2 responses as in Text-fig. 17 A, but in another experiment.

Text-fig. 18 B. Same experiment as Text-fig. 18 A, but for S responses.



Text-fig. 19.



Text-fig. 20.

Text-fig. 19. For S_1 responses as in Text-fig. 18 B, but in another experiment.

Text-fig. 20. The circles and the crosses show the testing preganglionic S_2 responses at the corresponding intervals after a maximal preganglionic and a maximal antidromic volley respectively, the ganglion having been painted with a 0·01 p.c. solution of nicotine about 25 min. previously. In addition to the curves through these points, the corresponding curves before the action of nicotine are also shown, the continuous lines showing the antidromic curves and the broken lines the preganglionic curves (cf. Text-figs. 17 A and 18 A).

two depressions. This transient facilitation suggests that an antidromic impulse sets up c.e.s. in the ganglion cells and that the initial period of increasing depression is really due to the more rapid decay of the facilitatory effect that opposes the more long-lasting depression, which itself actually reaches a maximum much earlier than is indicated by the composite curves of Text-figs. 17 A, 18 and 19.

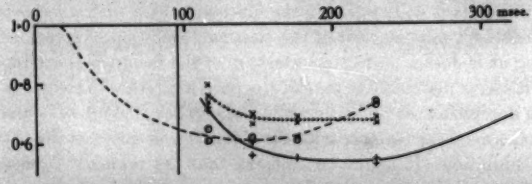
The action of small doses of nicotine in removing the phase of increasing S_2 inhibition at the same time as the S_1 facilitation indicated

such a solution for the S_2 inhibition set up by a preganglionic volley [Eccles, 1935 *b*, section L]. Similarly the period of increasing depression set up by an antidromic volley is removed by small doses of nicotine (Text-fig. 20), the latter parts of the curves simultaneously obtained after preganglionic and antidromic volleys being much less affected. This confirms the suggestion that the short period of increasing depression after an antidromic volley is also due to the more rapid decay of a small facilitatory effect set up by the antidromic volley. Further confirmatory evidence is provided below by a study of the interaction of preganglionic and antidromic volleys.

Thus the long-lasting depression produced by an antidromic volley is indistinguishable from the so-called inhibition set up by a preganglionic volley, and, as it will appear in the discussion that such a depression is responsible for part at least of the classical inhibition of spinal reflexes, forming as it does a perfect counterpart of the facilitation produced by c.e.s., it seems justifiable to extend the term inhibition to cover the long-lasting depression set up by an antidromic volley, though of course such an application is at variance with the classical conception of the nature of reflex inhibition. It might be objected that the normally interpolated facilitation provides an entirely arbitrary separation of a continuous prolonged depression into an earlier phase which has been regarded in section B as a refractory state, and a later phase now regarded as inhibitory in nature. In fact Hughes & Gasser [1934 *b*] and Lorente de Nó [1935 *b*] prefer to call the entire period of depression a relatively refractory period. However, though it is undoubted that the refractory state overlaps the inhibition, the following evidence clearly distinguishes between them in the ganglion cell. Nicotine in suitable doses abolishes the prolonged depression, i.e. the inhibition, but leaves a short period of depression which is presumably the true refractory period. Further, the prolonged depression (inhibition) is closely associated with the *P* wave which indicates that a maximum is only reached after about 20 msec. (cf. Eccles, 1935 *c*, Text-fig. 9), while, if the depression of excitability due to a refractory period is associated with any potential, it presumably would be the negative potential of the spike as may occur in peripheral nerve [Gasser & Graham, 1932]. Thus the prolonged depression (inhibition) appears to be more closely related to the subnormal excitability and the associated positive after-potential of peripheral nerve [cf. Eccles, 1935 *c*, p. 498] than to its relatively refractory state.

The interaction of two preganglionic volleys on the excitability of the ganglion cells was tested by a third preganglionic volley at various

intervals after the second [Eccles, 1935 *b*, section K]. The interaction of a preganglionic volley with a later antidromic volley has been similarly investigated, and in Text-fig. 21 the points show the potentials evoked by the testing preganglionic volley plotted against the respective intervals after the initial preganglionic and antidromic volleys. The control curves for the inhibitory effects of these volleys alone are also drawn. The inhibitory effect of the initial preganglionic volley is diminished for as long as 100 msec. after the intercurrent antidromic volley, an effect apparently corresponding to the period of increasing inhibition set up by the antidromic volley alone. This indicates that this period of increasing inhibition is due to the rapid decay of an antagonistic process



Text-fig. 21. The perpendicular line at 96 msec. shows the time of an antidromic volley following an initial preganglionic volley, and the oblique crosses show the S_2 potentials evoked by a testing preganglionic volley (expressed as a fraction of the normal control response) at four intervals later. The circles and the upright crosses respectively show the testing preganglionic responses after the preganglionic and antidromic volleys alone, the remainders of the corresponding curves being drawn from the observations made a little previous to those giving the plotted points.

which almost certainly is identical with the c.e.s. set up by a preganglionic volley, for both processes are also similar in their time course and in their sensitivity to nicotine. At all intervals after the antidromic volley the inhibitory effect of the combined preganglionic and antidromic volleys is less than that of the antidromic volley alone. This intense occlusion of the antidromic inhibition by the pre-existent preganglionic inhibition unmasks the effect of the c.e.s. which is set up by the antidromic volley and which is usually submerged beneath the more intense inhibition.

The interactions between the effects of two antidromic volleys or of an antidromic with a later preganglionic volley on the excitabilities of the ganglion cells resemble that between a preganglionic and a later antidromic volley (typically shown in Text-fig. 21), and in addition all these three types of interaction are similar to that described in section K [Eccles, 1935 *b*] for the interaction of two preganglionic volleys; hence a

general similarity of the effects of preganglionic and antidromic volleys is indicated. From all the experimental evidence stated in this section it may therefore be concluded that an antidromic volley resembles a preganglionic volley in setting up both the excitatory and inhibitory states of ganglion cells, c.e.s. and c.i.s. The antidromic c.e.s. is always much less than that set up by a preganglionic volley, but with c.i.s. there is no such large deficiency, the antidromic c.i.s. being perhaps at times even greater than that set up by a preganglionic volley.

An attempt has been made to determine the effect of an interpolated antidromic impulse on the facilitation between two preganglionic volleys. There is no doubt that some facilitation, and hence presumably c.e.s., is not inactivated by the antidromic volley, but interpretation of results is complicated because the c.e.s. and c.i.s. produced by the antidromic volley are affected by the pre-existent c.e.s. and c.i.s. Such interactions seem adequate to explain the action of the interpolated antidromic volley without assuming that it inactivates c.e.s. Such an explanation would conform with the absence of any apparent action of an antidromic volley on the preganglionic *N* wave (section B).

Discussion

(1) *The path of an antidromic impulse*

It has been previously pointed out that the histological picture of a nerve cell suggests that an antidromic impulse traverses the surface of the cell body and the dendrites to their terminations [Eccles & Sherrington, 1931 *b*]. While this suggestion is not at present susceptible to direct proof [cf. Forbes, 1934], the following evidence proves that in some nerve cells at least the antidromic impulse penetrates as far as the locus at which impulses normally are set up by a nerve cell.

(1) When an antidromic impulse is backfired into a rhythmically discharging soleus motoneurone, it not only is followed by a cycle longer than the normal cycle, but it also permanently alters the phase of the rhythmic discharge [Eccles & Hoff, 1932].

(2) If in the flexor reflex an antidromic impulse is interpolated between two centripetal volleys, there is a diminution of any facilitation which the first may exert on the second [Eccles, 1931].

(3) An antidromic impulse backfired into a motoneurone sets up a refractory state of that motoneurone which is not simply an absolute block, for the motoneurone exhibits a raised neurone threshold during a

period corresponding to a relatively refractory state [Eccles, 1931; Lorente de Nó, 1935 *b*].

(4) Similarly in the ganglion cell an antidromic impulse sets up a relatively refractory state during which the threshold for the discharge of an impulse is raised [Brown, 1934; section B above].

(5) When allowance is made for the different temporal dispersions, the ganglionic spike potentials set up by maximal antidromic and preganglionic volleys seem similar (section A), and the spike potential set up in the ganglion by a preganglionic volley is abolished when an antidromic volley prevents the setting up of a discharge by that volley (section B), thus again showing that an antidromic volley does not prevent the discharge by setting up a block peripheral to the point of origin of that discharge.

It may, therefore, be concluded that an antidromic impulse backfired into a nerve cell traverses the same path in that cell as an impulse normally set up by that cell and discharged along its axon. Hence it seems likely that an impulse discharged from a nerve cell has exactly the same action on that cell as an antidromic impulse, for in peripheral nerve the effect of an impulse is independent of the direction of its travel. An investigation of the effects of antidromic impulses on the rhythmic discharge from soleus motoneurones [Eccles & Hoff, 1932] lends experimental support to this suggestion, which is now to be examined in the light of the observations of this paper.

(2) N *wave and facilitation*

A preganglionic volley has actions on a ganglion cell other than the setting up of an impulse and actions secondary to this impulse, for it is established that it may give rise to an excitatory state and the associated *N* wave even when no impulse is discharged (section B) [Eccles, 1935 *b*, *c*]. Such additional effects produced by a preganglionic volley provide at least a partial explanation of the differences between the actions on ganglion cells of antidromic and preganglionic volleys, even if the discharge of an impulse actually has itself the same action as an antidromic impulse, for it has been shown that a maximal preganglionic volley sets up a larger *N* wave (section A) and facilitation (section D) than a maximal antidromic volley.

(3) P *wave and inhibition*

With regard to the *P* wave and inhibition the evidence is uncertain, for there usually are discrepancies between the respective series of observations in both of the following respects.

(1) The *P* wave set up by a maximal antidromic volley is always smaller and often much smaller than that set up by a maximal preganglionic volley (Text-fig. 3; Pl. I, fig. 2), while the intensity of the antidromic inhibition is never much smaller and often is as large or a little larger, even at such long intervals that the complicating effect of the more intense preganglionic facilitation must have passed off (Text-figs. 17 A and 18 A). This discrepancy is illustrated in Pl. III, fig. 10, and in Text-fig. 17. Since the antidromic slow positivity is considerably distorted by the stimulus artefact, the course of the slow positivity of both the initial preganglionic and antidromic responses has been determined in Text-fig. 17 B by plotting against the stimulus intervals the corresponding increases in the slow negativities of the subtracted curves of the testing preganglionic responses, a procedure which has been justified in section C (3). Except at short intervals, where the larger preganglionic *N* wave interferes, the preganglionic slow positivity is much greater than the antidromic slow positivity, hence the preganglionic *P* wave is also much greater. But for these same observations the preganglionic inhibition is smaller (Text-fig. 17 A), and the complicating effect of the more intense preganglionic facilitation certainly seems to have passed off at the longest intervals.

(2) When set up either preganglionically or antidromically, the *P* wave usually decays more rapidly than the inhibition. It was suggested [Eccles, 1935 *c*] that such a discrepancy could perhaps be due to an effect on *P* by amplifier distortion or tissue polarization, but such suggestions are of course invalid when an identical course for the *P* wave is indicated by the increase of the slow negativity set up by a testing preganglionic volley. Moreover, this discrepancy is sometimes absent or even slightly in the reverse direction [Eccles, 1935 *c*, Text-fig. 20], variations which are inexplicable by the above suggestions.

Hence it must be concluded that the *P* wave and the inhibition do not usually run identical time courses, and that, relative to the effect of a preganglionic volley, an antidromic volley sets up a *P* wave proportionately smaller than its inhibitory effect. This conclusion of course does not signify that these two reactions are absolutely distinct activities of the ganglion

cell, for in many ways they are closely related. To the evidence on this point already adduced from the action of a preganglionic volley [Eccles, 1935 *c*, Discussion] must now be added the similar evidence adduced in this paper both from the action of an antidromic volley and from all possible interactions of antidromic and preganglionic volleys (sections C and D), e.g. the *N* and *P* wave interaction of Pl. II, fig. 7, is very similar to the interaction of facilitation and inhibition in Text-fig. 21.

Evidence has already been brought forward [Eccles, 1935 *c*, Section F] which suggested a hypothetical *P* process of the ganglion cell which gave rise to the potential change (presumably in the surface membrane) which is recorded as the *P* wave. The evidence strongly suggested that this hypothetical process was also the basis of the raised threshold of the ganglion cell, which is of course indicative of c.i.s., i.e. that the *P* process was identical with c.i.s. With the technique at present available for investigating the living ganglion cell we can only determine its responses in terms of changes in its threshold and in an electrical potential which, on analogy with peripheral nerve and other isolated tissues that have been investigated, is largely if not entirely produced across its surface membrane. It is important to regard such changes as no more than special aspects of fundamental reactions of the cell. Complicating factors may prevent such a fundamental reaction from being expressed to a comparable degree in two aspects such as the *P* wave and the raised threshold. For example, the coexistent fundamental reaction (hypothetical *N* process or c.e.s.) which probably similarly underlies the *N* wave and the lowered threshold of facilitation may diminish the expression of the hypothetical *P* process or c.i.s. in terms of the raised threshold more than in terms of the *P* wave, hence the explanation of the discrepancy illustrated in Text-fig. 17, for more of the hypothetical *N* process or c.e.s. is set up by a preganglionic volley than by an antidromic volley.

Hence it may be concluded that there is no reliable experimental evidence suggesting that a maximal preganglionic volley sets up less c.i.s. than a maximal antidromic volley. Now it has been shown that the discharge of an impulse by a ganglion cell probably inactivates some of the c.e.s. of that cell [Eccles, 1935 *b*, section E], an action which undoubtedly is to be correlated with the production of c.i.s. by a preganglionic volley, i.e. a preganglionic volley gives rise to c.i.s. by an action secondary to the impulses which it sets up, and this c.i.s. may not be less than that set up by an antidromic volley. Thus the experimental evidence qualita-

tively supports and quantitatively does not contraindicate the original suggestion that an antidromic impulse has the same action on a ganglion cell as an impulse discharged by that cell.

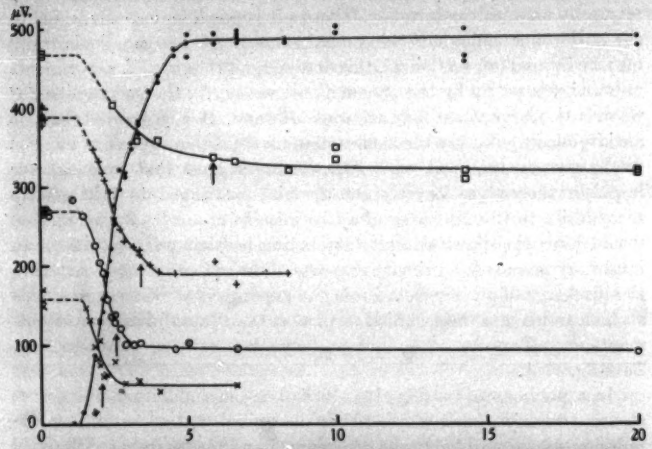
Specific inhibitory preganglionic fibres?

In some experiments, e.g. Text-fig. 18 A, it seems that a maximal preganglionic volley sets up a more intense inhibitory effect than can be set up by an antidromic volley. Though it is possible that this is due to the antidromic volley not being maximal, e.g. the postganglionic trunk may be injured (cf. section A), this does suggest that in such experiments more c.i.s. is set up by the preganglionic volley, for the preganglionic *P* wave is also larger than the antidromic *P* wave. If it be granted that the antidromic impulse has the same action on a ganglion cell as an impulse discharged by that cell, such experiments suggest that preganglionic impulses themselves directly set up c.i.s. additional to that set up secondarily to the discharge of an impulse from a cell. Such impulses would have the classical inhibitory action on that cell, setting up an inhibitory process as a primary response of the cell and not as a sequel to the discharge of an impulse. From the experimental evidence available such an action must be regarded as no more than doubtful in some experiments, e.g. Text-fig. 18 A, and probably not existing in others, e.g. Text-fig. 17 A.

In a previous paper [Eccles, 1935 *b*] specific inhibitory fibres were assumed to exist, for it was not then recognized that an antidromic impulse (and hence probably the discharge of an impulse from a cell) could set up such an intense long-lasting depression of excitability. However, none of the evidence presented in that paper proves that any of the inhibition is produced by specific inhibitory fibres, for even the almost complete restriction of the inhibition to the S_2 ganglion cells can be explained by the preponderating facilitatory type of response of the S_1, S_3 and S_4 ganglion cells, and the larger *P* wave (and hence probably c.i.s.) produced in S_2 ganglion cells both by antidromic volleys (cf. Text-fig. 2) and preganglionic volleys [cf. Eccles, 1935 *c*, Text-figs. 3, 4 and 5], though of course the reason for this unique behaviour of S_2 ganglion cells is still obscure. Again none of the experimental observations on the slow potential waves set up by preganglionic volleys [Eccles, 1935 *c*] proves that the *P* wave is set up by specific preganglionic fibres, *e.g.* as the intensity of the preganglionic stimulus was varied, the size of the *P* wave closely paralleled the size of the S_1 and S_2 spike potentials, the apparent absence of a *P* wave with very weak S_1 responses probably being

due to the preponderating N wave set up under such conditions [cf. Eccles, 1935 *c*, Text-figs. 3, 4 and 5].

The question of the existence of the classical inhibitory fibres may be investigated by a development of the procedure adopted in testing for the threshold of the inhibitory preganglionic effect [Eccles, 1935 *b*, Pl. III, fig. 9, and section I]. An initial (inhibitory) preganglionic volley



Text-fig. 22. In each of the series of observations (shown by the squares, upright crosses, circles, and oblique crosses) the potentials of the postganglionic S_2 responses evoked by a constant testing preganglionic stimulus are plotted as ordinates against as abscissæ the strengths of a varied preganglionic stimulus applied at a constant interval previously. The strength of the second preganglionic stimulus is shown for each series by the arrows immediately below the curve. The dots show the potentials of the S_2 responses evoked by the first stimuli plotted as ordinates against the corresponding stimulus strengths.

is set up by a stimulus of varied strength and at the same electrodes a second (inhibited) volley of constant size is set up at a fixed interval later, this stimulus interval being made longer than the optimal interval for inhibition in order to avoid complications arising from the c.e.s. which is of course also set up by the first volley. In Text-fig. 22 the potentials of the inhibited S_2 responses (recording as in Text-fig. 1 B) are plotted as ordinates against as abscissæ the strengths of the corresponding first preganglionic stimuli for each of the four series of observations, the

strength of the second preganglionic stimulus, which was constant for each series, being indicated by the arrow immediately below the curve drawn through the points of the corresponding series. In addition the curve expressing the relation between the potentials of the initial S_2 responses against the corresponding stimulus strengths is also drawn [cf. Eccles, 1935*c*, Text-figs. 3, 4 and 5], and close to zero stimulus strength are plotted the S_2 potentials evoked by the second volley alone for each of the series of observations.

The interpretation of Text-fig. 22 depends upon the following argument. As the first preganglionic stimulus is strengthened to equal eventually the constant second stimulus (submaximal) for that series of observations, the field of ganglion cells excited to discharge impulses by the first volley will increase *pari passu* until it becomes identical with the field responding to the second volley alone, for both volleys should then be identical, being set up as they are by identical stimuli applied through the same electrodes. Any further increase in the first volley will excite in addition a discharge from ganglion cells whose inhibition is not being tested by the second volley, for inhibition is only indicated by a diminution in the number of ganglion cells responding to this testing volley. Thus if the inhibition of the response to the second volley is solely due to the depression of excitability resulting from the discharge of impulses set up by the first volley, there should be, as the first stimulus is strengthened to equal the second, a progressive increase in the inhibition to a maximum which is not altered by any further increase in the first stimulus.

Actually, however, the following three factors would combine to delay the attainment of such a maximum until the first stimulus is slightly stronger than the second, for only then would the first stimulus set up a discharge from the whole field of ganglion cells excited by the second volley.

(1) There are probably small spontaneous variations in the excitability of the preganglionic fibres similar to those described by Blair & Erlanger [1933, 1936].

(2) Again there are presumably also small spontaneous variations in the thresholds of the ganglion cells.

(3) When preceded by the first volley, the second volley may set up the discharge of impulses from additional ganglion cells on account of a small facilitatory effect surviving during the long stimulus interval.

There is no way at present of determining the magnitude of these three effects, and it seems doubtful if they would be sufficiently large to

account for the maximum inhibition not being attained until the first stimulus is about 20 p.c. stronger than the second, as is shown in Text-fig. 22, which is typical of the series of observations in the four other experiments. Failing that explanation the presence of specific inhibitory fibres provide the only conceivable mechanism by which a delay in the attainment of a maximum could be brought about, the thresholds of some of these fibres being higher than those of the excitatory preganglionic fibres for the field of ganglion cells investigated. Thus the existence of specific inhibitory preganglionic fibres must be left undecided, but on account of the closeness with which the size of the first response parallels the inhibition of the second (Text-fig. 22), and of the magnitude of the antidromic inhibitory action, it must be concluded that such fibres, even if they exist, exert a much smaller inhibitory effect than that produced secondary to the discharge of impulses by the ganglion cells.

The relationship between antidromic and preganglionic impulses

There is more definite evidence that the *P* wave is to some extent directly set up by preganglionic impulses. Thus the *P* wave set up by a preganglionic volley is probably always larger than that set up antidromically (Text-fig. 3), and, when the refractory period following an antidromic volley prevents a preganglionic volley from setting up a discharge from the ganglion cells, the preganglionic volley still produces an additional *P* wave (Text-fig. 10). This special action of preganglionic impulses does not of course imply the existence of specific preganglionic fibres (which presumably would be of the classical inhibitory type), for the additional *P* wave could be produced directly by the ordinary excitatory preganglionic impulses. However, on account of the larger *N* wave directly set up by a preganglionic volley, the discharge of an impulse by a ganglion cell may itself give rise to a larger *P* wave than an antidromic impulse, for the *P* wave set up by either an antidromic or a preganglionic volley is increased by a pre-existent *N* wave (section C (4)).

Thus it must be concluded that the evidence suggests, but does not prove, that preganglionic impulses directly set up a *P* wave, which as we have seen is probably one aspect of c.i.s., a fundamental response of the ganglion cell. If preganglionic impulses do thus directly set up c.i.s., the direct actions of preganglionic and antidromic impulses on ganglion cells are qualitatively similar, both setting up c.e.s. and c.i.s., but quantitatively they differ,

for a preganglionic impulse directly sets up an intense c.e.s. and no more than a weak c.i.s., and an antidromic impulse (and probably the discharge of an impulse from a ganglion cell) an intense c.i.s. and a weak c.e.s.

General discussion

In this section experimental observations on the action of antidromic impulses on other nerve cells will be considered in the light of the conclusions reached in regard to ganglion cells. In section A, Umrath's results [1933] have suggested that antidromic impulses probably set up *N* and *P* waves in any nerve cell, and presumably such waves indicate the existence of the corresponding fundamental reactions, c.e.s. and c.i.s.

The motoneurones of the flexor reflex. In the original investigation on antidromic impulses any late depression of the excitability of motoneurones corresponding to Umrath's slow positive wave could not have been detected, for no examination of excitability was made at intervals beyond the stage of apparently complete recovery from the refractory period set up by that volley [cf. Eccles, 1931, Text-figs. 2 A, B]. It is now evident that such an apparently complete recovery probably was due to a temporary balance of the c.e.s. and c.i.s. set up by the antidromic volley (cf. Text-fig. 18 B). Such an interpretation has the further advantage of providing an explanation of the removal of facilitation by an interpolated antidromic volley [cf. Eccles, 1931, Pls. 39 and 40, figs. 3, 4 and 5] without drawing the conclusion (as was then done) that an antidromic volley inactivated c.e.s., an action which it does not appear to have on ganglion cells or on the motoneurones of the third nerve [Lorente de Nó, 1935 *b*]. Thus on analogy with the behaviour of ganglion cells a pre-existent *N* wave of the motoneurones would cause an antidromic volley to set up a larger *P* wave and probably a smaller *N* wave (cf. Text-fig. 16), effects which presumably also would be reflected in the corresponding c.i.s. and c.e.s. production with the result that there would be the apparent inactivation of the pre-existent c.e.s. when sampled by a later testing centripetal volley. In 1931 the existence of such an effect was of course not suspected, and its presence would not be indicated by the control observations on the action of the antidromic volley on the testing volley alone.

Motoneurones of the crossed extensor reflex. The action of an antidromic impulse when backfired into a rhythmically discharging motoneurone receives a more complete explanation than has hitherto been possible. Each discharge of the normal rhythmic series would normally set up a short *N* and a longer *P* wave whose course delays the setting up

of the next discharge and so conditions the duration of the normal cycle. An interpolated antidromic impulse would add to the P wave and so delay the subsequent discharge. The maximum beyond which the P wave (and presumably the c.i.s.) of the ganglion cell cannot be increased provides an analogy to the basal level beyond which it was impossible to depress the motoneurone [cf. Eccles & Hoff, 1932]. Moreover, the short N wave and c.e.s. presumably set up by a normal discharge (as well as by an antidromic impulse) provides a possible explanation of the hitherto inexplicable grouped discharges of motoneurones which so often appear after abnormally long cycles or at the beginning of a tetanus.

Motoneurones of the third cranial nerve. Lorente de Nó [1935 *b*] has shown that an antidromic volley sets up a depression of excitability of these motoneurones for as long as 15 msec., and he regards this as analogous to the late subnormality of peripheral nerve rather than to the classical refractory period (cf. section D). Moreover, such neurones differ from ganglion cells in showing no interpolated period of facilitation. He also concludes that an antidromic volley has no effect on pre-existent facilitation other than by the depression of excitability that it produces, the motoneurone thus resembling the ganglion cell; however, many of the observations supporting this conclusion have been complicated by the antidromic impulses being blocked from reaching many motoneurones on account of the impulses discharged from these motoneurones.

Inhibition. The probable absence of specific inhibitory fibres to the superior cervical ganglion raises the question of their existence in the spinal cord, for the inhibitory phenomena of the ganglion closely resemble those of the flexor reflex [cf. Eccles & Sherrington, 1931 *a*, *c*], though in the flexor reflex a volley exerts an inhibitory action on motoneurones without setting up the discharge of an impulse from them. However, Hughes & Gasser [1934 *b*] have presented a scheme by which this could occur through action on internuncial neurones, which histological observations [Hoff, 1932] and the experimental observations of Lorente de Nó [1935 *c*] have shown probably to be interpolated in the central pathway of the flexor reflex. It seems certain that part at least of reflex inhibition in the spinal cord is due to the depression of such nerve cells secondary to the discharge of impulses, for Hughes & Gasser [1934 *b*] find that the time course of the inhibition of the intermediary potentials resembles the positive wave. Moreover, there is as yet no proof of the existence of specific inhibitory fibres in the spinal cord, though Kato's experiments [1934] certainly suggest their presence in the frog. However that may be, the inhibitory state of ganglion cells, which is

largely if not entirely set up in the absence of specific inhibitory fibres, seems to be analogous to the inhibitory state exhibited by neurones of the spinal cord—at least in the case of the flexor reflex—for in all respects it forms a counterpart to the c.e.s. of ganglion cells, which undoubtedly is analogous to the c.e.s. of the flexor reflex.

Summary

An antidromic volley backfired into the superior cervical ganglion gives rise to the following changes in the ganglion cells.

1. A large spike potential, which suggests that an antidromic impulse traverses the ganglion cell and possibly the dendrites.

2. An absolutely refractory period of no more than 2 msec. and 3 msec. for the fastest S_1 and S_2 ganglion cells respectively. This is followed by a relatively refractory period, which shows that an antidromic impulse must penetrate as far as the locus at which the ganglion cell discharge is set up.

3. A slow potential wave, which analysis by the action of nicotine and by the interaction of antidromic and preganglionic volleys shows to be compounded of a negative wave *N* running concurrently with a larger and longer positive wave *P* (ganglion positive to its axon).

4. A prolonged depression of the excitability of the ganglion cells (as tested by a preganglionic volley), which analysis by the action of nicotine and by the interaction of antidromic and preganglionic volleys also shows to be compounded of a short facilitatory effect submerged beneath a longer period of depression.

The interaction experiments show that the antidromic *N* and *P* waves are indistinguishable from those waves set up by a preganglionic volley, and in addition confirm the previous findings on the interaction of *N* and *P* waves, *i.e.* pre-existent *P* in excess of *N* increases the *N* and diminishes the *P* wave set up either by an antidromic or preganglionic volley, and conversely pre-existent *N* in excess of *P* increases the production of *P* and decreases *N*. Similarly the facilitation and depression of excitability of the ganglion cells set up by an antidromic volley are shown to be qualitatively similar to the facilitation and inhibition set up by a preganglionic volley.

A maximal antidromic volley always sets up a much smaller *N* wave and facilitation than a preganglionic volley, but the *P* wave may be almost as large and the depression of excitability (inhibition) is often larger than that set up preganglionically. Despite the absence of a complete parallelism between the *P* wave and the depression of excitability of the ganglion

cells it is still thought that these reactions are aspects of the same fundamental state of the ganglion cells, which may be called the *P* process or c.i.s. Similarly the *N* wave and the facilitatory effect are thought both to be aspects of the fundamental state, c.e.s.

The experimental evidence is regarded as indicating that an antidromic impulse has the same action on a ganglion cell as an impulse discharged by that cell, the much larger *N* wave and facilitation set up by a preganglionic volley being due to the direct action of preganglionic impulses on the ganglion cells. On the other hand most of the *P* wave and inhibition produced by a preganglionic volley are set up secondarily by the discharge of impulses by those cells. The evidence is against the existence of specific inhibitory preganglionic fibres, but the excitatory preganglionic fibres in addition to their indirect action may also directly set up some *P* wave and c.i.s.

The action of antidromic impulses on other nerve cells and inhibition in the spinal cord is discussed in the light of these results.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

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Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

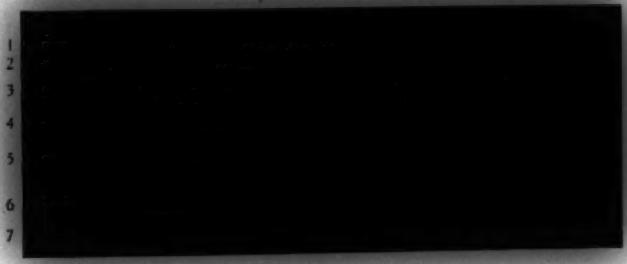

Fig. 10.

EXPLANATION OF PLATES I–III

Plate I

Fig. 1. Ganglionic action potentials evoked by an antidromic volley (observations 1 and 3) or by a preganglionic volley (observation 2), the amplification with observation 3 being 2·7 times that of observations 1 and 2. With an upward deflection in this and all subsequent records the ganglion is negative to the postganglionic trunk. In all figures 1 d.v. equals 10 msec.

Fig. 2. As in fig. 1, but at a much slower speed so as to show the full course of the slow potential waves set up by an antidromic volley (observations 2 and 4, stimulus strengths 20 and 14·3 arbitrary units respectively), and by a preganglionic volley (observations 1 and 3, stimulus strengths 10 and 20 arbitrary units). The antidromic stimulus of 20 units was almost maximal, for strengthening it by 50 p.c. only slightly increased the spike and did not alter the positive wave, which in all observations is the slow downward deflection.

Fig. 3. As in fig. 3, but with weaker stimulus strengths—0·67 for observation 1; 1·0 for 2; 1·25 for 3; 1·67 for 4; 3·3 for 5; 2·0 for 6.

Fig. 4. Observations 1 and 2 as in fig. 2, but observations 3 and 4 were taken about 3 min. after painting the ganglion with 0·005 p.c. nicotine, and observations 5 and 6 after a further painting with 0·05 p.c. nicotine. Observations 1, 3 and 6 show the responses elicited by a maximal preganglionic volley, and observations 2, 4 and 5 by a maximal antidromic volley.

Plate II

Fig. 5. Action potentials produced in the ganglion by maximal antidromic, *A*, and preganglionic, *P*, volleys at the following intervals—1, *P* alone; 2, *A* 2·5 msec. *P*; 3, *A* 0·0 msec. *P*; 4, *P* 11·9 msec. *A*; 5, *A* 11·4 msec. *P*; 6, *A* alone; 7, *A* 4·0 msec. *P*.

Fig. 6. Ganglionic action potentials produced by two maximal antidromic volleys at the following intervals—observation 1, 43 msec.; 2, 227 msec.; 3, 326 msec.; 4, 151 msec.; 5, control of second alone.

Fig. 7. Ganglionic action potentials produced by a maximal preganglionic volley followed by a maximal antidromic volley at the following intervals—observation 1, control of *A* alone; 2, 43 msec.; 3, 108 msec.; 4, 217 msec.; 5, 299 msec.; 6, 147 msec.

Plate III

Fig. 8. Ganglionic action potentials produced by a maximal antidromic volley followed by a maximal preganglionic volley at the following intervals—observation 1, control of *A* alone; 2, 0·0 msec.; 3, 147 msec.; 4, control of *P* alone; 5, 309 msec.; 6, 199 msec.; 7, 110 msec.; 43 msec.

Fig. 9. As in fig. 7, but for submaximal preganglionic and antidromic volleys (almost maximal for S_4) at the following intervals—observation 1, 231 msec.; 2, 338 msec.; 3, 148 msec.; 4, 21 msec.; 5, *A* alone; 6, *P* alone; 7, 77 msec.

Fig. 10. Ganglionic action potentials evoked by a submaximal preganglionic volley (control response in observation 1) at intervals of 143, 87 and 231 msec. respectively after either a maximal preganglionic volley (observations 3, 5 and 6) or a maximal antidromic volley (observations 2, 4 and 7).

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CARBAMINO COMPOUNDS OF CO_2 WITH HUMAN HÆMOGLOBIN AND THEIR ROLE IN THE TRANSPORT OF CO_2

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(Received May 4, 1936.)

Introduction

A chemical method for the estimation of CO_2 bound to hæmoglobin by a carbamino linkage, i.e. bound to an $—NH_2$ group on the hæmoglobin molecule, was described by Ferguson & Roughton [1934*a*]. In their succeeding paper [1934*b*] evidence was presented that the carbamino compound must play a significant part in the transport of CO_2 by the blood. Only ox hæmoglobin was used in these experiments as difficulty was anticipated in applying the method to the hæmoglobin of most other animals. A tentative calculation, however, of the role of carbamino compound in the transport of CO_2 by human blood was made by combining the data obtained on ox hæmoglobin with the data of L. J. Henderson and his associates on the conditions of CO_2 transport in human blood.

It was obviously desirable to investigate directly, as soon as possible, the capacity of human hæmoglobin to form carbamino compounds. Furthermore, certain points connected with the method had, for lack of time, received rather summary investigation and obviously deserved further attention.

The work to be reported in this paper was undertaken with these two considerations in view and its scope may be outlined as follows:

(1) The chemical method of estimating carbamino CO_2 has been modified for use with solutions of human hæmoglobin.

(2) A more ambitious attempt has been made to estimate the loss of carbamino CO_2 during a determination. As a result it has become evident that the previous allowance for such loss was considerably too low.

(3) The utility of the chemical method has been further extended by the demonstration that it is applicable to solutions with low total CO_2 content. (The validity of the method under such conditions was previously under some suspicion.)

(4) The effect of varying pressures of CO_2 on the carbamino CO_2 content of solutions of human hæmoglobin, with base added to simulate conditions within the red blood corpuscle, has been investigated at CO_2 pressures ranging from 4 to 80 mm. Hg, and with the hæmoglobin fully oxygenated and fully reduced.

(5) To obtain data under conditions more nearly simulating those in the circulating blood, the effects of varying degrees of partial oxygenation of the hæmoglobin on the formation of carbamino compound have been determined.

(6) Finally, a simplified method, applicable under limited conditions, is proposed for the estimation of the importance of carbamino compound in the transport of CO_2.

Nomenclature

The combination of CO_2 with an $—NH_2$ group on the hæmoglobin molecule may be represented by the equation

$$CO_2 + HbNH_2 \rightarrow HbNHCOOH.$$

Hæmoglobo-carbamic acid was suggested by Ferguson & Roughton as a name for the carbamic acid so formed.

At hydrogen-ion concentrations which obtain in the red blood corpuscle, this acid would be largely dissociated to form $HbNHCOO^-$ [Stadie & O'Brien, 1935], and in this state would be referred to as hæmoglobo-carbamate. These names are particularly useful in discussion which requires that the ionized and un-ionized states be distinguished. For many purposes, however, it would be more convenient to use a shorter name to designate the total carbamino compound, i.e. ionized plus un-ionized. The name carbhæmoglobin [Henriques, 1928] would be eminently suitable and has been used in this sense by Groscurth & Haveman [1935]. It must be pointed out, however, that the indirect method of estimation employed by these authors cannot be regarded as measuring only carbamino-bound CO_2, but must include CO_2 or $\overline{HCO_3}$ bound to Hb by other linkages.

In this paper, carbhæmoglobin, represented by the symbol $HbCO_2$, will be used to mean total carbamino-bound CO_2 as measured directly by the chemical method.

Section I. Experimental methods and controls

The hæmoglobin solutions were prepared by the method of Adair [1934]. The stroma was separated by the use of purified ether and salt. The excess of salt and ether was removed by dialysis. It was found that dialysis against 0·5 p.c. KCl instead of distilled water helped to keep the loss of oxygen capacity of these solutions at a minimum during the time which must elapse before they could be used up. The solutions were kept frozen at about −3° C. in an electric refrigerator till they were used. In the week which usually elapsed before one preparation could be used up the loss in O_2 capacity did not amount to more than about 5 p.c.

The method of analysis for $HbCO_2$ will be outlined briefly, with detail sufficient only to make clear the modifications which have been introduced.

Combined CO_2 other than $HbCO_2$ is precipitated as $BaCO_3$ by the addition of alkaline $BaCl_2$. The $BaCO_3$ is removed by centrifuging. The procedure is to mount three 5 c.c. syringes (one containing the solution to be analysed and the two others the reagents) in a water thermostat, with their plungers resting on a movable platform. When the platform is elevated the three syringes are emptied simultaneously and their contents are expelled through a Hartridge-Roughton rapid mixer. The mixed fluid is caught in a centrifuge cup surrounded by ice. The precipitate of $BaCO_3$ is removed by centrifuging at 2000 r.p.m. for 10 min. The supernatant liquid is then analysed for CO_2 by the van Slyke method. The result gives the uncorrected or gross $HbCO_2$, which may be expressed as c.c. of CO_2 per 100 c.c. of original hæmoglobin solution.

The main difficulty encountered in applying the method to solutions of human hæmoglobin was a great exaggeration of the difficulty previously encountered in solutions of ox hæmoglobin containing low concentrations of total CO_2; namely, the "protective action" of the protein which hindered the complete separation of the $BaCO_3$ precipitate by centrifuging. In the case of ox hæmoglobin the difficulty has been overcome by increasing the bulk of the precipitate by adding sufficient $NaHCO_3$ with the reagents to bring the total CO_2 content of the system to 50 mM. per litre of original hæmoglobin solution. With human hæmoglobin, eight or ten times as much carbonate had to be added and even this amount was not as effective in securing complete removal of the $BaCO_3$ as the smaller quantity had been in ox hæmoglobin.

The usual blank correction for $BaCO_3$ remaining in the supernatant fluid after centrifuging was, for human hæmoglobin, 1 c.c. of CO_2 per 100 c.c. original hæmoglobin solution as against 0·2–0·4 c.c. for ox hæmoglobin.

It might be conjectured that the great bulk of precipitate used for human hæmoglobin would drag down a considerable amount of adsorbed hæmoglobin on centrifuging. Actually any such tendency proved to be negligible, for colorimetric estimation of the hæmoglobin in the supernatant fluid showed it to be present in exactly one-third the concentration of the original solution.

One preparation of human hæmoglobin was found to retain its protective action in spite of high concentrations of added carbonate. It has not yet been determined whether this was a personal characteristic, or whether a variation in the method of preparation was responsible.

Reagents for human hæmoglobin.

Human hæmoglobin was found to stand alkali in amounts sufficient for the determination without detectable destruction. It was found satisfactory for the purposes of the analysis to add to the hæmoglobin solution an equal volume of NaOH solution of a strength (in mol./litre) given by the formula: $[NaOH] = 12[Hb] + [CO_2]$.

$[Hb] = O_2$ capacity of the Hb solution in mol./litre. $[CO_2]$ = total CO_2 content of the Hb solution in mol./litre.

The carbonate for increasing the bulk of the precipitate was placed in the same syringe as the alkali. Since its concentration was large compared with variations in the CO_2 content of the hæmoglobin solution, it was always used in syringe C at a constant concentration of about 0·4 mol./litre.

TABLE I. Contents of syringes for the analysis of $HbCO_2$ in human hæmoglobin solutions

Syringe	Content
A	Hb solution to be analysed.
B	$BaCl_2$ (0·6 mol./litre).
C	$NaOH + Na_2CO_3$ (for concentrations see text).

Corrections

The analysis of the supernatant fluid gives a figure for the gross $HbCO_2$ (expressed as c.c. of CO_2 per 100 c.c. of original Hb solution), and to this figure the following corrections must be applied to obtain the preformed $HbCO_2$ in the original hæmoglobin solution. To correct:

(1) For dissolved CO_2 which turns into $HbCO_2$ on the addition of alkali, subtract 10 p.c. of the dissolved CO_2 in the original hæmoglobin solution.

(2) For incomplete removal of $BaCO_3$ by centrifuging, subtract 1 c.c. of CO_2 per 100 c.c. original hæmoglobin solution.

(3) For loss of $HbCO_2$ by dissociation during the determination, multiply the figure remaining after the two previous corrections have been deducted by 1·5.

The first two corrections are adequately discussed in the previous publication [Ferguson & Roughton, 1934*a*]; the last is new and requires attention in detail.

The loss of $HbCO_2$ *during an estimation*

Ferguson & Roughton found that prolonging the centrifuging for a second period of fifteen minutes resulted in a loss of only about 8 p.c. of the $HbCO_2$. This figure was used as a minimum correction for loss of $HbCO_2$ during the estimation. It was realized that the loss might be considerably greater, particularly in the interval after mixing, when (1) the mixture was not yet chilled sufficiently to stabilize the $HbCO_2$ and when (2) the bulk of the precipitate was not yet removed by centrifuging.

It seemed desirable to investigate the rate of dissociation of $HbCO_2$ in the presence of the alkaline $BaCO_3$ at different temperatures in the hope of making a more precise estimate of the loss of $HbCO_2$ which might occur during a determination.

The hæmoglobin solution was mixed with an equal volume of sodium carbonate solution (0·4*M*) with NaOH of the strength used in an ordinary determination. The flask was kept for twenty minutes in a water bath at the required temperature. Under these conditions (*p*H *ca.* 11–12, and high carbonate concentration) considerable amounts of $HbCO_2$ were formed. $BaCl_2$ solution was now added from another flask which had been standing in the same bath. The mixture was poured into six test-tubes, also standing in the bath. One tube was then centrifuged immediately (at a temperature as close as possible to that of the bath) and the remainder at appropriate intervals thereafter. Immediately before it was to be centrifuged each tube was inverted ten times to stir up the heavy precipitate.

On the addition of the $BaCl_2$ the concentration of dissolved carbonate and, *a fortiori*, the pressure of CO_2 in the mixture would become vanishingly small. Accordingly the CO_2 combined to hæmoglobin would, as it dissociated off, be removed immediately by hydration (carbonic anhydrase being active in the solution), and precipitated as carbonate. As each tube was centrifuged in turn, the $HbCO_2$ remaining in the supernatant fluid was found to be progressively less. Thus a curve could be plotted

showing the rate of dissociation of $HbCO_2$ at that particular temperature and in the mixture used in an ordinary analysis. Fig. 1 shows three such curves at the temperatures 1·5, 18 and 37° C. respectively.

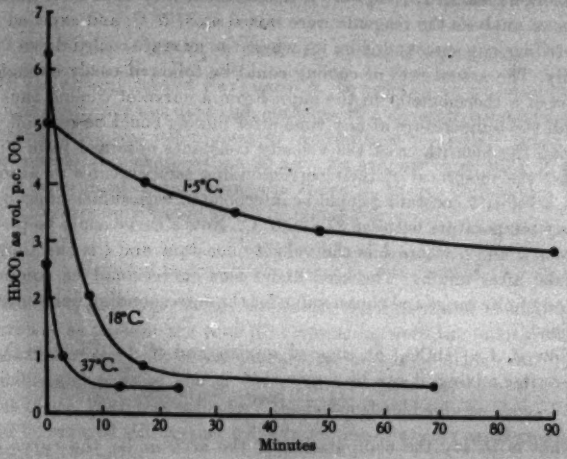

Fig. 1. Rate of dissociation of $HbCO_2$ in alkaline $BaCl_2$ at different temperatures (ox Hb).

These curves show a number of points of interest. Firstly, so far as they have been investigated they can be fitted by equations of the monomolecular type. Consequently the rate of dissociation at any time can be represented by a differential equation of the type

$$\text{Rate of reaction} = \frac{d[HbCO_2]}{dt} = k\,[HbCO_2],$$

t = time in seconds.

Secondly, the variation of k (the velocity constant) with temperature does not quite obey the Arrhenius equation. That is:

$$\ln k \neq \frac{C}{T},$$

T = absolute temperature.

This is not strange because the molecular species dissociating is, according to theory, HbNHCOOH (hæmoglobo-carbamic acid), and not $HbCO_2$ which is the entity measured by the analysis. The concentration of HbNHCOOH would not likely be the same fraction of the total carbhæmoglobin at different temperatures.

Thirdly, the rate of dissociation, particularly at the higher temperatures, proved to be greater than had been expected. For example at 37° C. the time of half completion was about 3 min. This velocity would allow considerable loss of $HbCO_2$ were it not checked very soon after mixing.

In an analysis the reagents were mixed at 37·5° C. and expelled into a centrifuge cup surrounded by ice where the mixture cooled down fairly rapidly. The actual rate of cooling could be followed easily enough by means of a thermometer in the cup. From a curve of cooling thus obtained, the temperature at any time after mixing could be read off. By plotting the logarithms of the velocity constants obtained from Fig. 1 against the reciprocal of their corresponding temperatures in absolute units, a velocity constant k could be interpolated with sufficient accuracy for any temperature between 37 and 0° C. Now a curve could be plotted between k and t, where k is the velocity constant and t is the time (in seconds) after mixing. The area under this curve could be computed over any finite range and thus a value for the corresponding finite integral obtained.

Now if $A=[HbCO_2]$ at time of mixing and if $A-X=[HbCO_2]$ at time t after mixing, it can be shown that

$$ln \frac{A}{A-X}=\int_{t=0}^{t=t'} k ds.$$

That is to say the computation of the area under the curve of k plotted against t enables the calculation of the loss of $HbCO_2$ during the interval from $t=0$ to $t=t'$.

The time t' is the time required to balance the centrifuge cups, to set the centrifuge in motion, and to separate the bulk of the precipitate. It was not very easy to evaluate this time precisely but 90 sec. may be considered a fair average value for t'. (Loss during the remaining 9 min. of centrifuging has been neglected. 90 p.c. or more of any carbonate formed by the dissociation of carbhæmoglobin after the separation of the bulk of the precipitate would remain in the supernatant fluid adsorbed to the protein. The further centrifuging serves only to pack down the precipitate.)

When $t'=90$ sec. the graphical integration of the curve gives a value of ln 1·5 for the expression $ln \frac{A}{A-X}$. Therefore $A=1·5\,(A-X)$. In other words the approximate correction to be applied for loss during a determination is 1·5.

The curves shown in Fig. 1 are for ox hæmoglobin. It was not practicable to obtain satisfactory curves on human hæmoglobin at 37° C. because of the rapidity with which it is denatured by the alkali at that

temperature. At lower temperatures, however, the velocity constants were found to be of about the same magnitude for human as for ox hæmoglobin when the strength of alkali added to each was that used in ordinary determinations on each.

Loss of $HbCO_2$ *in previously published experiments*

In the experiments of Ferguson & Roughton on ox hæmoglobin, an angle centrifuge was used and, consequently, accurate balancing of the centrifuge cups was not necessary. Accordingly the time t' must have been considerably shorter than 90 sec. An average value for t' in those earlier experiments might have been about 25–30 sec. With such a value for t' the correction factor would be about 1·2.

Means of increasing the precision of the method

The foregoing experiments emphasize the importance of knowing as accurately as possible the time (t') elapsing between the mixing and the separation of the bulk of the precipitate. If a large angle centrifuge is available and handily placed, the separation time can be shortened to about 15 sec. With this value for t' the factor should only be about 1·1. It could be reduced still further by the adoption of a more rapid method of cooling the mixture.

Solutions with low total CO_2 *content*

On the basis of some rather complex control experiments which gave ambiguous results with solutions of low total CO_2 content Ferguson & Roughton did not feel justified in claiming that the method was applicable to such solutions. The question was whether the extra carbonate added in the rapid mixer was effective in causing the centrifuging down of carbonate which had been previously molecularly dispersed in the hæmoglobin solution, particularly if this previously dispersed carbonate were small in amount, in which case it might be the more completely surrounded by adsorbed protein. The problem was to obtain a low concentration of carbonate molecularly dispersed in the hæmoglobin solution but unaccompanied by any $HbCO_2$. The clue to obtaining the latter condition is given in Fig. 1. In the presence of alkaline $BaCl_2$ at 37° C. the $HbCO_2$ would almost entirely dissociate in a few minutes leaving only carbonate molecularly dispersed in the hæmoglobin solution. The effectiveness of carbonate added in the rapid mixer would be de-

monstrated if, after centrifuging, the supernatant fluid contained only minimal amounts of CO_2.

Experimental. Hæmoglobin solutions containing 2–10 vol. p.c. of total CO_2 were mixed with alkaline $BaCl_2$ and kept at 37° C. for 30 min., in which time practically all of the $HbCO_2$ would have dissociated. To the mixture was now added (in the rapid mixer) a solution of Na_2CO_3 of the strength used in an ordinary analysis. The precipitate of $BaCO_3$ was then immediately removed by centrifuging as in an ordinary analysis.

On analysing the supernatant fluid for CO_2, a typical experiment gave a value of 0·4 c.c. CO_2 per 100 c.c. original hæmoglobin solution. When water was added in the rapid mixer, instead of Na_2CO_3, nearly all of the carbonate previously present remained in the supernatant fluid after centrifuging.

Apparently then, the method can be used with confidence for solutions containing low concentrations of total CO_2.

Section II. The physiological importance of carbhæmoglobin

Total CO_2 *and* $HbCO_2$ *dissociation curves in solutions of human hæmoglobin*

Experimental. The hæmoglobin solutions used had as a rule an oxygen capacity of 20–22 vol. p.c. By the addition of Na_2CO_3 they were adjusted so that at a pressure of CO_2 of 40 mm. Hg they would have a *p*H of 7·1–7·3. This range may be regarded as including the normal range of *p*H in red blood corpuscles under resting conditions, at that pressure of CO_2. The approximate *p*H of the solutions was calculated by the Henderson-Hasselbalch formula using 6·1 arbitrarily as the value of pK_1'.

For the determination of one set of curves about 50 c.c. of solution were adjusted to the appropriate content of base and were used up within three days. To prevent deterioration the solution was kept on ice all day and frozen each night.

One set of curves is intended to show the effect of varying CO_2 pressures on the $HbCO_2$ content and total CO_2 content of the solution with the hæmoglobin fully reduced and fully oxygenated. In each set of curves it was practicable to obtain points at four or five different pressures of CO_2 and the range covered was from 4 to 80 mm. Hg. About 7 c.c. of solution were placed in a tonometer immersed in a water thermostat at a temperature of 37·5° C. and there equilibrated with a mixture of CO_2 and air (for oxygenated solutions) or with CO_2 and N_2 for reduced solutions.

The gas and liquid phases were separated by the method of Austin *et al.* [1922]. About 5 c.c. of the solution were drawn into syringe A to be analysed for $HbCO_2$; the remainder was analysed for total CO_2 and O_2 in the van Slyke apparatus. A sample of the gas phase was analysed for CO_2 in the Haldane apparatus.

Results.. Fig. 2 shows one set of curves; total CO_2 and $HbCO_2$ dissociation curves on oxygenated and reduced hæmoglobin. The data for two other full sets and a number of partial sets at different values of *p*H support the same general conclusions and are given in Table II.

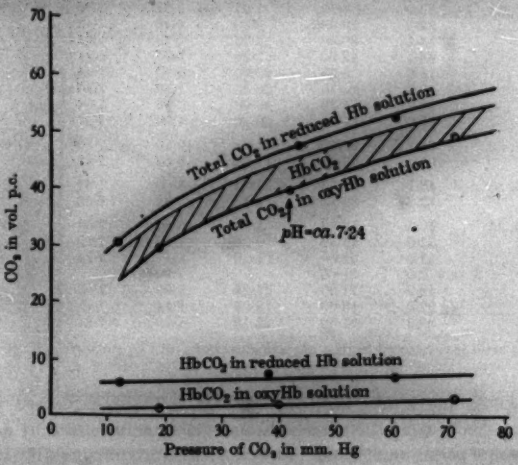

Fig. 2. Change in total CO_2 and $HbCO_2$ with changing P_{CO_2} in a solution of human Hb.

As in the previous work on ox hæmoglobin it was found that at the same pressure of CO_2 the reduced hæmoglobin solution invariably contained more $HbCO_2$ than did the oxygenated solution. The shaded area between the total CO_2 dissociation curves of oxy- and reduced hæmoglobin represents the proportion of the extra total CO_2 capacity of reduced hæmoglobin, which can be accounted for by the greater carbhæmoglobin content of the reduced hæmoglobin solution. It can be seen from Fig. 2 that 70–75 p.c. of the extra total CO_2 capacity (Haldane effect) can be so accounted for.

A very important characteristic of the carbhæmoglobin dissociation curves is that they are practically flat over a considerable range of CO_2

TABLE II

Date	Total CO_2 in vol. p.c.	Pressure of CO_2 mm. Hg	O_2Hb as vol. p.c. O_2	Corrected $HbCO_2$ vol. p.c.	Calculated pH	$\frac{HbCO_2}{O_2Hb}$*
26. v. 35	37·4	51·0	19·1	0·2	7·12	−0·25
	45·7	50·0	0·6	4·8	7·17	
24. viii. 35	49·5	31·5	1·30	6·16	7·41	−0·24
	54·4	41·8	1·41	5·70	7·33	
	34·8	13·9	1·33	4·50	7·62	
	58·4	58·5	1·27	7·20	7·20	
	42·8	30·3	19·20	1·77	7·41	
	53·6	60·3	19·00	2·10	7·19	
23. x. 35	15·9	4·45	1·57	2·31	7·78	−0·16
	32·2	19·60	0·80	4·45	7·43	
	58·7	80·50	1·08	4·48	7·08	
	45·7	43·00	1·20	5·05	7·24	
	15·6	8·28	20·60	0·90	7·54	
	27·9	21·30	20·80	1·86	7·36	
	48·7	74·20	19·00	2·86	7·04	
	39·0	45·30	20·30	1·92	7·17	
4. xii. 35	65·2	33·50	1·54	10·10	7·50	−0·33
	61·5	40·00	21·60	3·52	7·43	
5. xii. 35	43·1	59·70	21·24	3·00	7·07	−0·24
	49·0	38·40	1·18	7·80	7·30	
8. xii. 35	30·6	12·30	1·92	5·80	7·59	−0·27
	52·6	60·50	0·96	6·90	7·13	
	47·0	42·00	1·20		7·24	
		38·00		7·00		
	49·0	71·00	21·24	3·00	7·06	
	29·3	19·00	22·05	1·14	7·45	
	38·9	41·60	22·15	1·40	7·21	

* This ratio is calculated for the physiological range of CO_2 pressure, viz. 40–60 mm. Hg.

pressure. As previously pointed out, this is to be explained by the opposing effects exerted on the carbhæmoglobin equilibrium by a rise of CO_2 pressure *per se*, and the rise in acidity which accompanies it. In these solutions the opposing effects appear to cancel each other rather exactly over a range of CO_2 pressures from 15 to 80 mm. Hg. It seems then that over the physiological range of CO_2 pressure for resting conditions, the $HbCO_2$ dissociation curves may be regarded as practically flat. That is to say, in these solutions variations in $HbCO_2$ concentration are due almost entirely to changes in the degree of oxygenation of the hæmoglobin rather than to changes in CO_2 pressure.

Effect of partial oxygenation on the formation of $HbCO_2$

Claims for the physiological importance of carbhæmoglobin must be based largely on the effect of oxygenation on carbhæmoglobin formation. In calculating the probable role of $HbCO_2$ in CO_2 transport it has been

tacitly assumed that at intermediate stages of oxygenation of the hæmoglobin the concentration of $HbCO_2$ would be linearly proportional to the concentration of reduced hæmoglobin. So far, direct investigation has been made only on the effects of complete oxygenation and complete reduction. It seemed desirable to test the effect of intermediate degrees of oxygenation.

Experimental. About 50 c.c. of hæmoglobin solution with an appropriate amount of Na_2CO_3 added were divided into seven portions. Each was equilibrated at 37° C. with the same pressure of CO_2 (40 mm. Hg) but with different pressures of O_2. The solution was then analysed for total CO_2, O_2, and $HbCO_2$.

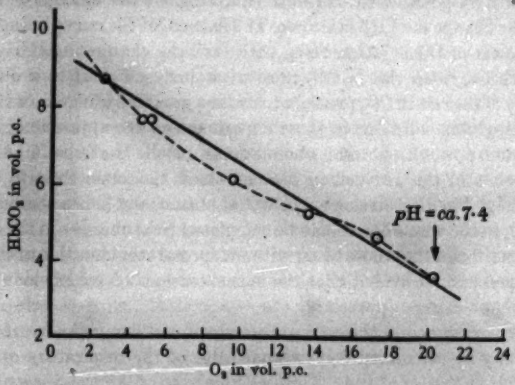

Fig. 3. Change in $HbCO_2$, with varying oxygenation of the Hb at constant P_{CO_2} (human Hb).

Results. Fig. 3 shows the concentration of $HbCO_2$ plotted against the oxyhæmoglobin concentration. The data suggest a slightly inflected curve rather than a straight line, but the deviations from a straight line are not beyond experimental error. Apparently then, it is permissible to assume a linear relation between the $HbCO_2$ content and the degree of oxygenation of the hæmoglobin. It may be expressed thus:

$$[HbCO_2] = C - K\,[O_2Hb],$$

and therefore

$$\frac{d\,[HbCO_2]}{d\,[O_2Hb]} = -K.$$

To put it verbally, the absorption by the hæmoglobin of a given volume of oxygen results in the expulsion of a definite volume of CO_2 from combina-

tion as carbhæmoglobin; the amount being determined by the magnitude of K.

For the data of Fig. 3 with concentrations expressed as vol. p.c., $C = 9{\cdot}6$ and $K = 0{\cdot}3$. An average value for K, for human hæmoglobin, from experiments on six different samples of human hæmoglobin at pH's ranging from 7·2 to 7·4 (at 40 mm. Hg pressure of CO_2) is about 0·24. This constant may be used to calculate the role of $HbCO_2$ in the transport of CO_2 by human blood.

The role of carbhæmoglobin in CO_2 transport

Because of the flatness of the $HbCO_2$ dissociation curves in these solutions it is possible to calculate (knowing K) the change in $HbCO_2$ if only the change in O_2Hb is known. The flatness of the curve is due to the buffer power of the solution being such that the change in acidity associated with a given rise in CO_2 pressure is just sufficient to nullify the tendency of the rise in CO_2 pressure to cause a greater formation of $HbCO_2$. The hæmoglobin solutions in these experiments have approximately the same buffer power as normal whole human blood. It seems fair then to assume that in the red cell in normal blood the same flatness of the carbhæmoglobin dissociation curve would obtain, and hence that changes in $HbCO_2$ in the whole blood may be calculated from changes in the degree of oxygenation of the whole blood without special consideration of changes of CO_2 pressure, provided that the concentrations of other acids in the blood do not change appreciably.

Under resting conditions then, the role of carbhæmoglobin transport of CO_2 may be calculated from a knowledge of the respiratory quotient for any short period of time. Thus with an R.Q. of 0·8 the absorption of 10 c.c. of O_2 is accompanied by the output of 8 c.c. of CO_2. But the combination of 10 c.c. of O_2 with hæmoglobin will simultaneously expel 2·4 c.c. of CO_2 from combination as carbhæmoglobin. This will comprise

$$\frac{2{\cdot}4}{8{\cdot}0} \times 100 = 30 \text{ p.c. of the total } CO_2 \text{ evolved in the lungs.}$$

For the sake of precision it should be mentioned that not quite all of the 10 c.c. of O_2 absorbed in the lungs is combined with hæmoglobin, but that as much as 0·3 c.c. may remain in simple solution in the blood. This quantity, however, is scarcely significant in the present calculation.

Thus it seems probable that under resting conditions as much as 30 p.c. of the CO_2 transported by the blood, that is to say, evolved in the lungs, may be carried as carbhæmoglobin.

It is not yet possible to estimate with any confidence the importance

of carbhæmoglobin in conditions of exercise because of the complication introduced by the accumulation of lactic acid.

In the changes in CO_2 content of the red cells during the respiratory cycle carbhæmoglobin must play an even greater part than it does in the changes of the whole blood. Probably at least 70 p.c. of the transport of CO_2 by the red cells is accomplished by changes in the concentration of $HbCO_2$ in the cells.

Comparison of ox and human hæmoglobin

In solutions of reduced ox hæmoglobin at physiological *p*H and pressures of CO_2 of 40–60 mm. it was found that $HbCO_2$ comprised 8–10 p.c. of the total CO_2. With human hæmoglobin under the same conditions, $HbCO_2$ comprises 10–16 p.c. of the total CO_2. This greater apparent affinity of human hæmoglobin for CO_2 is due partly to the greater corrections for loss of $HbCO_2$ which have been used in calculating the results on human hæmoglobin. It was pointed out, however, in an earlier section of this paper that a comparable correction applied to the results previously reported for ox hæmoglobin would not increase the figures by more than 20 p.c. It seems evident then that many samples of human hæmoglobin really possessed a greater capacity than ox hæmoglobin to form carbhæmoglobin. The results on oxygenated hæmoglobin support the same conclusion. In oxygenated solutions of ox hæmoglobin $HbCO_2$ comprised 2–4 p.c. of the total CO_2. In oxygenated solutions of human hæmoglobin it comprises 2–7 p.c. These figures are, however, less reliable than those for reduced solutions because they are smaller and greatly affected by corrections.

The greater affinity of human hæmoglobin for CO_2 may be attributed to the greater buffer power of human hæmoglobin, as suggested to me in a personal communication by Dr F. J. W. Roughton. This greater buffer power implies:

(1) A greater number of free $—NH_2$ groups at a given *p*H, and hence more groups to react with CO_2;

(2) It also implies more base to neutralize hæmoglobo-carbamic acid as it is formed, with less change of *p*H;

and thus may act in one or both of these ways.

Carbhæmoglobin and former views of CO_2 *transport*

For many years now the greater CO_2 capacity of reduced blood has been ascribed to a difference in acid strength of the hæmoglobin in the reduced and oxygenated states. It might be timely to point out here that

the recent work on carbhæmoglobin in no sense refutes such a change in acid strength or its physiological importance. Although a large part of the extra combined CO_2 in the reduced hæmoglobin solution has now been shown to be combined directly to the hæmoglobin as $HbCO_2$ it must be borne in mind that this $HbCO_2$ is an acid (hæmoglobo-carbamic acid) which requires base to neutralize it no less than does carbonic acid. The base liberated when the hæmoglobin becomes a weaker acid on losing its oxygen enables hæmoglobo-carbamic acid to be neutralized without taking base from bicarbonate.

Not only does carbhæmoglobin fit into the classical picture of CO_2 transport with remarkably little disturbance to the classical design, but it has helped to explain some anomalies in the classical scheme. For example it has been known for some years [Roughton, 1935; Stadie & Hawes, 1928] that the apparent value for pK_1' of carbonic acid is different in the presence of reduced and oxygenated hæmoglobin. The anomalous change in pK_1' has been partly explained by the discovery of carbhæmoglobin.

Summary

1. The chemical method of estimating carbamino compounds of CO_2 with hæmoglobin is shown to be applicable to solutions of human hæmoglobin when suitable measures are adopted to overcome the great "protective action" of this protein on the precipitate of $BaCO_3$.

2. From measurements on the rate of dissociation of $HbCO_2$ in alkaline solutions at different temperatures, the loss of $HbCO_2$ during determinations under various conditions is calculated. Implications regarding the precision of the method are discussed.

3. The chemical method is shown to be applicable to solutions of low total CO_2 content.

4. Carbamino compounds of CO_2 with human hæmoglobin and ox hæmoglobin have the same general properties. One of the most important of these from the physiological point of view is that oxygenation greatly diminishes the affinity of hæmoglobin for CO_2.

5. Higher figures are reported in this paper for the amounts of CO_2 combined with human hæmoglobin than were previously reported for ox hæmoglobin under comparable conditions. Reasons for this are discussed.

6. A higher estimate, too, is made of the physiological importance of $HbCO_2$ in the transport of CO_2. It is calculated that about 30 p.c. of the total CO_2 transport at rest, and about 75 p.c. of the transport in the red cells is by $HbCO_2$.

It is a pleasure to acknowledge my indebtedness to the Banting Research Foundation for a grant for the purchase of apparatus; also to Prof. F. R. Miller and Prof. A. Bruce Macallum for generous facilities to pursue this research.

It is a pleasure also to acknowledge helpful criticisms of the manuscript by Dr F. J. W. Roughton.

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THE GENESIS OF RESPIRATORY MOVEMENTS IN THE FŒTUS OF THE SHEEP

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(*Received May* 4, 1936)

In a previous paper by Barcroft, Barron & Windle [1936] a description was given of the integration of the earliest individual movements into a mass movement. In the present paper that movement, as we understand it, will be described more particularly, and an account will be given of the derivation from it of movement of the type familiar in ordinary mammalian respiration.

The experimental procedure is as given in the paper quoted above. The mass movement is well seen in sheep embryos of 36–37 days (27–29 mm. crown-rump length), and may be evoked by touching the nose with a glass rod. This may be done, if the rod is a fine one, without letting any considerable quantity of fluid out of the membranes. Here as elsewhere, unless it is otherwise stated, the observations recorded are with the embryo inside the membranes. We were always careful to note the colour of the blood in the cord, and the results given apply to experiments in which that was satisfactory. The pulse in most cases was counted. The mass movements are seen under urethane anæsthesia or with the mother under a spinal anæsthetic.

The next stage in the development of the respiratory movement follows very shortly. The movement does not alter in type; instead of being a single mass reaction, evoked by a specific stimulus or perhaps two such reactions in quick succession, it becomes definitely rhythmic, a group of movements appearing and petering out; the rate of the rhythm being usually 30–45 to the minute, though the group of movements dies away after perhaps half a minute. The youngest fœtus in which we saw this phenomenon was aged 38 days and was 31 mm. in length.

"Ewe was under light urethane anæsthesia, 3 c.c. per kg. and the uterus delivered into the bath at 40° C." and the fœtus was exposed in the intact amnion. "There were no spontaneous movements until after the fœtus had been exposed some time.... The head appeared to be extended, the trunk straightened and the limbs and tail extended. These appeared rhythmically—a sharp contraction and rather a slow relaxation. On one occasion they were definitely initiated by extension of the hind foot of the left side with abduction of the toes. This rhythmic extension and relaxation of the leg occurred several seconds before the generalised extension took place."

The rhythm described above is spoken of as spontaneous. This description must not be insisted upon except to distinguish it from the movements previously described, which were elicited only as the result of the rather forceful application of a glass rod to the surface of the embryo. Actually the rhythm of which we are now speaking made its appearance shortly (perhaps half a minute, but the time was not regular) after the application of a glass plate to the surface of the amnion for purposes of photography. It seemed likely that the movements were caused by the presence of the plate. That they were due to associated thermal or luminary effects was ruled out, and it was probable, though not proven, that they were due to the pressure of the plate.

The rate of the "rhythm" bore no relation to that of the rhythm of the mother's respiration. Nor was it that of the fœtal pulse as is shown by the following table.

TABLE I

Serial No. of sheep	Fœtal age (days)	Rate of fœtal rhythm per minute	Fœtal pulse rate per minute	Anæsthetic administered to mother
66	40	33	164	Urethane
103	41	42	162	Spinal
61	42	20	84–128	Urethane
67	43	30	—	"
121	43	32*	126	Spinal
82	44	48	—	Urethane
85	45	36	—	"
122	45	44	120–124	Spinal
96	46	42	128	Urethane
100	48	66	192	Spinal

* Later 48 irregular.

It must provisionally be taken as accidental that in sheep 66, 103, 96 and 100, the figure of the respiratory rate is within the limits of counting an aliquot part of the pulse rate. The two were not counted simultaneously.

No one who has seen both can fail to be struck with the similarity between these mass movements and the gasps which occur at the late stages of poisoning by inhalations of minimal lethal doses of hydrocyanic acid vapour.

The conversion of this rhythmic mass movement into movements of a more normal respiratory type is simply a process of "whittling" down.

In general the extensor movements go before those of the flexor type. The mouth ceases to open rhythmically. The neck movements undergo an interesting change about the 40th day. At first the head is simply thrown back during each mass movement. The question of the mechanism we leave open. Possibly both flexor and extensor muscles are involved and that the observed extension is the algebraic sum of their effects. Or it may be that extensor muscles only are involved. But on the 41st day, as was pointed out to us by Dr Windle, the head movement is a diphasic one, an extension of the neck succeeded by a flexion.

The next stage is for the extensor phase to drop out, leaving only the flexor phase. The method of disappearance of the former is interesting. It does not fade out by becoming progressively less powerful, but appears intermittently and at rarer intervals.

"*Sheep No.* 110—41 *days—length* 40 *mm.* Immediately (on opening uterus) rhythmic movements were seen.... Each movement involved a flexion of the neck, but occasionally this was preceded by an extension of the neck. Sometimes there would be two, sometimes three flexions without extension to each flexion. The extension seemed sharper than the flexion."

Thus the general rhythm is never broken. These movements were particularly well seen in sheep No. 66, age 40 days, in which rhythmic movements of the diaphragm were also well marked as an associated part of the general rhythm. (Subsequent dissections showed that the diaphragm is complete on the 39th day.) These movements of the diaphragm of course mean that the ribs are drawn in and the liver depressed.

"*Sheep No.* 61, *age* 42 *days.* The embryo was executing rhythmic movements: they involved descent of the liver, opening of the mouth, flexion of the head and contraction of the infrahyoid muscles, as in panting."

Between the 40th and 45th days the active limb movements disappear also and the legs, in so far as they do move, only do so passively as the result of the trunk movements.

By the 46th day all rhythmic spasms have become reduced to movements of the trunk only. By this time the diaphragm is fully formed, and perhaps the most obvious phenomenon is the rhythmic depression of the liver due to the contraction of the diaphragm. The ribs are drawn in, presumably from this same cause, the chest being of course closed and the walls soft. In appearance the fœtus differs little from that of an ordinary animal which is breathing except for the drawing in of the ribs as described.

Spontaneity of the rhythmic movements

We have already stated that when first seen the movements seemed to depend upon some as yet ill understood stimulation of the fœtus. Up to the 49th day it was, however, more and more easy to elicit the rhythmic movements, so long as the fœtus was in good condition, and indeed they were elicited so easily on that day that it was very difficult to abolish them completely. The embryo presented the appearance of an animal which was breathing continuously; but the more quietly the preparation was held the slower and shallower the rhythm. The question naturally arose: if at this stage the embryo were not interfered with at all would it display rhythmic movements of the respiratory type?

To test the matter more closely without opening the uterus, we transilluminated it with a single light. Under these circumstances it was possible not only to see the shadow of the fœtus, but also its movements. At times these were certainly rhythmic, but even under these circumstances it cannot be claimed that the fœtus was entirely undisturbed; usually some manipulation of the uterus was required to bring the shadow into a position in which it was best seen. It must be remembered, on the other hand, that the fœtus is small relatively to the sac of fluid which contains it, and within the limits imposed by the length of the umbilical cord was free to move about much like a fish in a bowl.

The relation between the rhythmic movements and other muscular movements

For the most part, in what we have said above we have been dealing with movements associated with the minimum degree of interference to the fœtus. We observed incidentally that on the 49th day the more quietly we held the fœtus the slower and shallower was the rhythm. The reverse was true: muscular movement was associated with an increase both in frequency and power of the rhythm. Not only so, but the movement became more extensive, the head flexed and the infrahyoid muscles contracted. In sheep No. 101 when breathing forcibly the mouth also opened, whilst when making gentle respiratory movements it remained shut. The mass movement has been shorn of the head and limb movements between the 40th and 49th day: these movements have not been lost, but they have developed along their own lines and can be evoked by an appropriate stimulus. In particular they have assumed a relation to gravity, so that on the 49th day, if the fœtus be on its side and be

given a sharp tap either on the nose or the tail region it will perform a movement which bears a general resemblance to an effort to get up. This development of righting movement will form the subject of a later paper by one of us [Barron]. We mention it here only to say that with it is associated the increase both in rate and power of the rhythmic movements, so that the appearance presented is that of an animal which makes an effort to rise from the ground and is out of breath as the result.

Causal relation between muscular effort and rhythmic movements

It would be natural to assume as a provisional hypothesis that the acceleration of the rhythmic movements was due to the effect on the brain of blood impoverished in oxygen or enriched in carbonic acid by the muscular effort. About this theory there are several difficulties. The first is that the effect on the respiration is immediate: the second that no proof exists of this assumed asphyxial condition of the blood: and the third that an asphyxial flow does not at this stage (i.e. up to the 49th day), elicit this rhythm.

The first statement rests on inspection of the fœtuses themselves and of the films which we have taken of the phenomena concerned. With regard to the second, it must not be forgotten that this fœtal circulation is peculiar: between the time that a sample of blood traverses one of the contracting muscles and the time it reaches the brain, it (or most of it) will have traversed the placenta, where it will have had every opportunity of losing any additional carbonic acid which it may have acquired in the muscles.

As regards the third point, we have on many occasions between the 40th and the 49th days stopped the circulation in the umbilical cord. This procedure has never elicited respiratory movements; usually it has had no effect other than to prejudice the nervous system and temporarily to slow the heart. Sometimes it has caused an extension (not rhythmic) of the limbs. Once on the 49th day it produced a rhythmic chewing movement, but never in our experience has clamping or tying the cord within the first 49 days elicited the rhythm of the respiratory type which forms so striking a feature of fœtal life at that time.

The accentuation of this rhythm contingent on muscular effort seems not to be associated with the chemical regulation of respiration but with the essential physiological structure of the nervous system. The reflex muscular effort and the respiratory rhythm having developed from the original mass movement are linked together. The phenomenon in ordinary

mammalian physiology which seems most closely allied is that described by Krogh & Lindhard [1913], who, in their classical paper, show that "at the commencement of heavy work...the rise in ventilation...is produced by irradiation from the motor cortex".

The cessation of the rhythmic movements

About the 50th day, one-third of the way through fœtal life, a great alteration takes place: the fœtus from being very active becomes inert and the phenomena which have been described are usually not visible. The essential nature of this change is a matter for future investigation: here it can only be said that the mechanism which has been elaborated is not abolished: it is only dormant. One way of revealing it is that of subjecting the fœtus to asphyxial conditions, as by pinching the umbilical cord for a short time.

The relation of the movements described in this paper to those observed by Snyder [1936] in rabbit fœtuses at term cannot at present be precisely stated, and depends upon the relative maturities of rabbit fœtuses at term (30 days) and 49 day sheep fœtuses. Anoxæmia appears to depress both. There is a similar difficulty in comparing our results with the instructive paper of Corey [1932].

Summary

1. The rhythmic trunk movements associated with ordinary respiration are developed between the 38th and 49th days of fœtal life in the sheep (gestation period about 157 days).

2. They are derived from a general mass movement of an extensor type by the dropping out of the movements of the head and limbs.

3. They cannot be elicited at this stage by asphyxial conditions of the blood.

4. Towards the 49th day their frequency becomes more rapid and they become more powerful as the result of muscular effort.

5. The above phenomenon seems to be of the same nature as the irradiation hyperpnœa of Krogh & Lindhard.

6. After the 50th day the rhythmic movements disappear as a spontaneous phenomenon but can be elicited by stoppage of the blood flow in the umbilical cord.

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DIETARY DEFICIENCY, NERVE LESIONS AND THE DENTAL TISSUES

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(*Received May* 7, 1936)

It has been previously reported that, in experimental animals, deficiency of vitamin A or carotene in the diet results, not only in hyperplasia of the gingival and subgingival tissues, with the subsequent supervention of periodontal disease [M. Mellanby, 1930], but also in degeneration of the sensory nerves of the teeth and jaws [M. Mellanby & King, 1934]. It had been suggested by E. Mellanby [1933, 1934] that the epithelial and nervous lesions observed in vitamin A deficiency were closely related and that the former might be due to removal of, or interference with, the normal trophic impulses. On this basis it was considered [M. Mellanby & King, 1934] that such a loss of neurotrophic control, due to the effects of the vitamin deficiency upon the nerve or nerve cell, might be partly responsible for so-called pyorrhœa alveolaris and other diseases of the teeth and jaws. If this were the case interference with the nerve supply, including experimental resection of the afferent fibres of the dental nerves, might also be accompanied by hyperplastic and other degenerative conditions of the periodontal tissues. In 1934 the experiments here described were begun with the object primarily of testing this point. It was, however, borne in mind that such operations would not necessarily reproduce alterations in nerve-cell function and structure similar to those observed in the metabolic derangement associated with a dietary deficiency of vitamin A.

In the following series of experiments upon dogs and rabbits, the effects of resection of certain nerves are discussed and compared with those due to vitamin A deficiency. In the course of the investigation it was found that the part played by the vaso-motor system in the growth of the teeth had also to be included.

Inferior dental nerve operations

After examination of the distribution of the sensory dental nerves in the animals to be used, it was decided that those supplying the lower jaw were the more suitable for operation. Eventually the inferior dental nerve was selected for experimental section, in spite of the fact that the periodontal tissues of the lower jaw are also supplied by the lingual and long buccal nerves. This additional nerve supply is in some respects a serious disadvantage, but by severing the inferior dental little or no damage is done to other tissues; moreover, this nerve appears to possess no motor fibres and so is not likely to affect mastication.

The inferior aspect of the mandible, below the roots of the cheek teeth, was chosen as the site of operation in both dogs and rabbits (see radiographs, Pl. I and Text-fig. 1); in puppies, below the second deciduous molar; in adult dogs, below the fourth premolar or first molar; and in rabbits, beneath the roots of the second premolar and first molar.

In all of these regions the under side of the mandible is free from muscular and tendinous attachments. Small branches of the inferior labial and submental arteries and of the facial vein, and cutaneous nerve terminations are the only structures, other than subcutaneous tissue, which are likely to be encountered between the skin and the periosteum. Relatively close to the under surface of the bone, and within its substance, lies the inferior dental canal containing vessels and nerve.

In this position, therefore, the inferior dental canal was opened and, with the aid of a blunt aneurism needle, carefully inserted between the nerve and artery, about three-quarters of an inch of the former was lifted and excised from one side of the lower jaw of a number of dogs and rabbits. In the dogs the effects of such lesions were also compared with those due to dietary deficiency.

Series I (Dogs)

With the exception of animals *a* and *b* (Table I), which were adults, the excisions were performed on all of the dogs at an early age and the effects of such treatment adjudged by observation of the condition of the permanent dentition. Some of the animals received diets adequate, and others deficient in vitamin A, as shown in Table I.

Age at beginning of the dietary period: 6–8 weeks.

Basal diet: white bread, 120 g.; lean meat, 20 g.; separated milk powder, 20 g.; bakers' yeast, 7·5 g.; sodium chloride, 1 g.; orange juice, 6 c.c.

The above results, together with the radiographs and other illustrations shown in Pls. I and II, may be summarized as follows:

(*a*) *Vitamin A deficiency.* The effects of deficiency of vitamin A upon the teeth and periodontal tissues of each dog were determined by the condition of the whole of the upper jaw and, as shown in Table I, by that of the *left* (*unoperated*) *side* of the mandible. In animals *b*, *e*, *f* and *g* (Pl. I and II), receiving diets deficient in vitamin A, there was on the whole an increased deposition of tartar about the necks of the permanent teeth, accompanied by varying degrees of gingivitis. As seen in Pl. II, figs. 2 and 4, dogs *b* and *g*, the alignment of the lower incisors was irregular, the latter perhaps being caused by crowding of the teeth due to deficient growth of the mandible. Radiographs of the lower jaws at death showed the roots of the cheek teeth to be imperfectly developed and the laminæ duræ not well defined; the bone trabeculæ in the immediate vicinity of the tooth roots appeared unusually opaque to X-rays in some regions (Pl. I, figs. 2 and 4). Histologically, degeneration of sensory nerves and hyperplasia and downward proliferation of the subgingival epithelium were characteristic defects; in addition, the abnormal radiographic appearance of the tooth roots was seen to be due to excessive deposition of cementum, which in some cases had invaded the apical portion of the pulp chamber (Pl. II, figs. 6 and 7). The addition of calcium carbonate to the ration of dog *g* (Pl. I, fig. 4; Pl. II, figs. 4 and 7) produced no obvious improvement in the periodontal tissues or in the alignment of the front teeth.

In contrast with the above, animals *a*, *c* and *d* showed much more normal conditions. The amount of gingivitis and cervical tartar was considerably reduced; the incisor teeth were in proper alignment; abnormalities of cementum and bone were absent; and the nervous and epithelial tissues presented a normal appearance (Pl. I, figs. 1 and 3; Pl. II, figs. 1, 3 and 5).

(*b*) *Experimental nerve lesions.* The effects due to resection of the right inferior dental nerve were assessed by comparing the *right side* of the lower jaw, peripheral to the site of operation, with the left (control) side. The most obvious result of the nerve lesion was the effect produced upon the alignment of the front teeth. Pronounced linguoversion of the second incisor, and to a lesser extent bucooversion of the third incisor, was observed even when the diet contained liberal amounts of vitamins A and D and other essentials (Pl. II, figs. 1 and 3, dogs *a* and *c*). In those animals receiving A-deficient rations, since lack of this vitamin was itself associated with similar conditions in the lower

TABLE I. The effects of vitamin A deficiency and of resection of the right inferior dental nerve upon the lower jaw and permanent teeth of dogs*

No. of dog	Dietary additions	Period on diet (months)	Age at operation (months)	Post-operative period (months)	Brief general clinical history	Irregularity of incisors at end of experiment†	
a‡	Cod-liver oil (vitamins A and D)	33	22	12	Active, normal	I_1 ... lv –	I_1 ... lv – I_2 ... lv + + I_3 ... bv –
b‡	Irrad. ergosterol (vitamin D) Peanut oil	25	11	11	Active but very incoordinate; cornea of each eye opaque and ulcerated, with some watery discharge; hair absent from chest and abdomen.	I_1 ... bv –, dv – I_2 ... lv + + +	I_1 ... bv –, dv – I_2 ... lv + + +
c	Irrad. ergosterol (vitamin D) Mammalian liver oil (Vitamin A)	6	2	6	Very active, normal	—	I_2 ... lv + + + I_3 ... bv +
d	Irrad. ergosterol (vitamin D) Mammalian liver oil (Vitamin A)	7	1½	7	Very active, normal	I_2 ... lv –	I_1 ... bv – I_2 ... lv + + I_3 ... bv +
e	Irrad. ergosterol (vitamin D) Peanut oil	7	1½	7	Active, slight incoordination of hindlegs.	I_2 ... lv – I_3 ... bv +	I_2 ... lv + I_3 ... bv +
f	Irrad. ergosterol (vitamin D) Peanut oil	7	1½	7	Active, somewhat incoordinate in hindlegs.	I_1 ... dv + I_2 ... dv –	I_1 ... dv + I_2 ... lv + I_3 ... bv + + +
g	Irrad. ergosterol (vitamin D) Peanut oil Calcium carbonate	5	2	5	Very weak and incoordinate; cornea of each eye opaque and ulcerated, with watery discharge.	I_1 ... bv – I_2 ... lv + + I_3 ... bv +	I_1 ... lv – I_2 ... lv + + I_3 ... bv +

* I am grateful to British Drug Houses, Ltd., for the vitamin A (mammalian liver oil) and vitamin D (irradiated ergosterol) used in these experiments.

† lv, linguoversion, inside normal line of occlusion; bv, buccoversion, outside normal line of occlusion; dv, distoversion, distal to normal position.

‡ Dog full grown at operation, all permanent teeth erupted except $\frac{M_3}{M_3}$.

N.B. In animal *d*, both lower first premolars were absent, and in animal *g* both the deciduous and temporary canines were present.

incisor region, the effects of the nerve operations cannot easily be judged (Pl. II, figs. 2 and 4, dogs *b* and *g*).

In the experiments described here no marked differences, macroscopic, radiographic, or histological, in the condition of the periodontal tissues were observed between the operated and control sides of the lower jaw, even in the incisor region. When the diets were deficient, the changes associated with lack of vitamin A were present; when there was an adequate supply of this vitamin, the tissues appeared normal in structure.

Although at first sight it might appear that the mechanism controlling the incisor misalignment, seen both in vitamin A deficiency and after experimental nerve excision, had a common origin, a consideration of the time of eruption of the permanent teeth of both sides of the lower jaw of two of the young operated dogs, receiving, respectively, diets adequate and deficient in vitamin A, indicated that the deformities arose from different causes, as shown in Table II.

TABLE II. The time of eruption (piercing of the gum) of the permanent lower teeth. The effects of experimental excision of part of the right inferior dental nerve compared with those of deficiency of vitamin A in the diet

Age at operation: 1½ months.
Postoperative period: 7 months.
Diet: as in Table I.

	Dog *d* (Diet containing vitamin A)			Dog *f* (Diet deficient in vitamin A)		
	Time of eruption (days after birth)			Time of eruption (days after birth)		
Perm. teeth	Unoperated side	Operated side	Difference (−early, +late) days	Unoperated side	Operated side	Difference (−early, +late) days
I_1	119	119	—	129	129	—
I_2	126	119	−7	136	136	—
I_3	136	133	−3	150	143	−7
C	147	140	−7	161	161	—
Pm_1	Absent	Absent	—	147	152*	+5
Pm_2	147	147	—	164	167*	+3
Pm_3	154	157*	+3	164	164	—
Pm_4	147	154*	+7	164	164	—
M_1	159	159	—	166	166	—
M_2	154	154	—	171	171	—
M_3	164	164	—	180	180	—

* It is probable that the tissues in the vicinity of these teeth may have been damaged during the operation.

This table allows a comparison to be made between the effect of vitamin A deficiency and that of the experimental nerve lesion upon the rate of eruption of the mandibular permanent teeth of dogs *d* and *f*.

In the first instance, on the unoperated side it is evident that the teeth of the vitamin A-deficient dog *f* erupted, on an average, about 13 days later than the corresponding ones of dog *d*, which received a liberal supply of vitamin A. The nerve operation, on the other hand, resulted in a slight acceleration of growth of the second or third incisor of the operated side, as compared with the corresponding teeth of the unoperated side of the same animal; it will be noted that the incisor teeth so affected were those which had previously been observed to be out of alignment (see Table I). The position of the canine of either dog, on account of the size of the tooth and the length of its root, would hardly have been expected to be much affected. The proximity to the site of operation of the premolars peripheral to the nerve lesion renders it inadvisable to draw any conclusions from the time of eruption of these teeth.

It would seem, therefore, that vitamin A deficiency and excision of the inferior dental nerve tend to have reverse effects upon the rate of eruption of the lower incisor teeth, although their end result is similar as regards tooth alignment. On this basis, however, it is difficult to account for the incisor irregularity produced in dog *a*, all of whose teeth were fully erupted prior to the nerve operation.

Series 2 (Rabbits)

Experimental removal of part of the right inferior dental nerve was also performed on eleven rabbits. Since the teeth of these animals grow from persistent pulps, by drilling small parallel holes in the cervical enamel of the incisor of each side of the lower jaw and observing the rate at which these holes approached the biting edge, it was possible to compare the rate of tooth growth on the operated and unoperated sides. This procedure was adopted in five of the operated rabbits and in seven unoperated controls. In all these animals the distance from the lower border of the drilled hole to the biting edge of the tooth was recorded at frequent intervals. In the other six operated animals, which were allowed to survive for 1–4 months after the nerve had been excised, the growth of the teeth was not estimated, the object in this instance being observation of any possible pathological changes in the teeth and periodontal tissues over a varying postoperative period.

The diets of all the animals, experimental and control, included an adequate supply of fat-soluble vitamins, being composed of: "nibbed" oats, 40 g.; wheat bran, 10 g.; heated alfalfa, 10 g.; calcium carbonate, 0·75 g.; lemon juice, 1 c.c.; vitamin D, 500 I.U.; and cabbage, 30 g. or

mammalian liver oil (vitamin A), 250 "blue" units. The average age of the animals at the time of operation was 3 months, when they were receiving 70 g. of the above diet daily.

Results. The effects of the operation upon the rate of growth of the teeth of rabbits can best be described in conjunction with the accompanying graphs (Text-figs. 5, 6 and 7), where AB represents the time

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

The effects of experimental resection of the inferior dental and cervical sympathetic nerves upon the rate of growth of the incisor teeth of rabbits. (See also p. 70, Text-figs. 5–10.)

Text-fig. 1. Radiograph of the lower jaw of a rabbit after excision of the right inferior dental nerve, showing the site of operation (O).

Text-fig. 2. Photograph of the incisors of control animal 6 (series 2), 17 days after holes had been drilled in the cervical enamel of the lower teeth, showing their normal rate of growth. No nerve operation had been performed.

Text-fig. 3. Photograph of the incisors of animal 1 (series 2), 17 days after resection of the right inferior dental nerve. Note the more rapid rate of growth of the lower tooth of the operated side (O).

Text-fig. 4. Photograph of the incisors of animal 19 (series 3), 18 days after resection of the right cervical sympathetic trunk. Note the more rapid growth of the tooth of the operated side (O).

after operation and BC the growth of the teeth during this period, the line passing horizontally through C being the level at which the drilled holes had grown out of the teeth. The following are typical of these experiments.

Animal 1 (Text-fig. 5). On the eighth day after operation it is seen that the right incisor had grown distinctly faster than the left (control). This acceleration of growth increased steadily until, on the twenty-first

day, the hole in its buccal surface was no longer visible. At this time the left incisor hole was still 2 mm. below the biting edge, which it passed on the thirtieth day after the operation. Text-fig. 3 illustrates the appearance of the lower incisors of this animal 17 days after resection of the right inferior dental nerve; the relative rate of growth of the teeth, as evidenced by the position of the holes in their buccal surface, is clearly indicated. Text-fig. 2 is a photograph of an unoperated control animal (No. 6) at a similar stage.

In animals 2 and 3 (Text-figs. 6 and 7), following acceleration in growth of the right incisor during the first 8 days after operation, the teeth of both sides grew at relatively similar rates until the twenty-first and eighteenth day respectively. In each animal the growth of the right tooth then became retarded, and the holes in all the teeth disappeared on the twenty-seventh day.

During the first 19 days after operation, the average rate of growth of the lower incisor teeth was approximately: on the operated side, 0·28 mm. per day; on the unoperated side, 0·21 mm. per day.

Control animals (*unoperated*) 6–12 (Text-fig. 2). The incisor of each side grew at a rate corresponding to that of the control teeth in the operated animals. This shows that the resection has been followed by accelerated growth. In some cases the increase in growth took place in the first week only and it appears that subsequently a slight retardation may sometimes occur. The weights of animals 1–5 fell for the first day or two after operation, but soon recovered and increased more or less consistently throughout the experiments.

Apart from the occasional presence of small, opaque, whitish patches on the enamel of both lower incisors, no other abnormal conditions were observed, clinically or radiographically, in the teeth or periodontal tissues of any of the animals in which the resection was done, even in those surviving for 4 months after operation. Histologically, increased vascularity of the tissues on the operated side of animals 1–5 was the only change to be noted in the teeth or surrounding structures peripheral to the nerve lesion. On the other hand, Astanin and Kauschansky [1931] claim to have produced "macroscopic caries" and gingivitis by severing the second or third divisions of the trigeminal nerve of rabbits; but since all the teeth of rabbits grow from persistent pulps, they can hardly be subject to typical dental caries.

Reverting to the growth-rate experiments, the rabbit findings confirm those of series 1 (dogs). It appears, however, unlikely that destruction of the sensory fibres of the inferior dental nerve would accelerate

tooth growth. It is of course possible that in rabbits the nerve lesion in some way affected the composition of the tooth, rendering it more or less

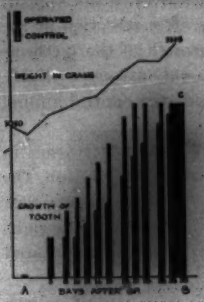

Fig. 5.

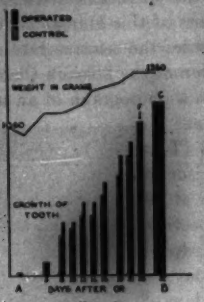

Fig. 6.

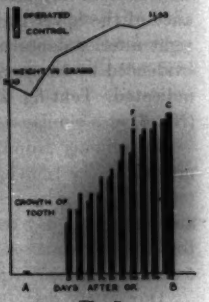

Fig. 7.

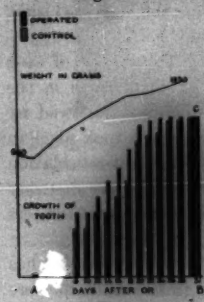

Fig. 8.

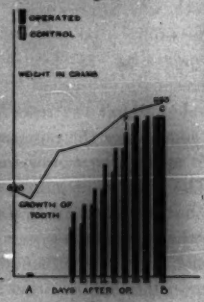

Fig. 9.

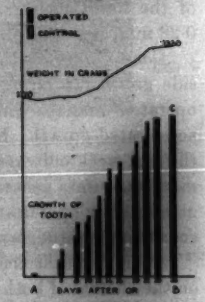

Fig. 10.

The effect of experimental resection of the inferior dental and cervical sympathetic nerves upon the rate of growth of the incisor teeth of rabbits.

Text-figs. 5–7 illustrate graphically the rate of growth of the lower incisors of animals 1–3 (series 2) after the inferior dental operation.

Text-figs. 8–10 illustrate the growth of the lower incisors of animals 19–21 (series 3) after the cervical sympathetic operation.

The distance $BC = 6{\cdot}5$ mm. in Text-figs. 5 and 6, and 6·0 mm. in Text-figs. 7–10; for further description of graphs see text, pp. 67 and 71.

brittle, but no such change was noted by the methods available. Certainly the incisal edge on the operated side must, if the tooth grew faster, have been more susceptible to attrition than that of the control tooth, otherwise it would have projected beyond the latter. That there was no

obvious difference in length between the two teeth is shown by Text-fig. 3. In any case such an argument has little or no bearing upon the dog experiments.

It seemed more probable that some other nerves, such as those controlling the blood supply to the teeth, may have been in such close proximity to the inferior dental nerve that they too were severed or injured during the operation. Vaso-constrictor fibres are said to accompany the peripheral nerves [Gilding, 1932], and if these had been destroyed, it might be expected that vaso-dilatation of the peripheral vessels, including those of the teeth distal to the lesion, by increasing the volume of blood available, would tend to accelerate the changes associated with tooth growth. It must be emphasized here that the inferior dental vessels themselves were not damaged by the operation in any of the dogs or rabbits.

It was therefore decided to remove part of the cervical sympathetic cord, believed to supply vaso-constrictor fibres to the teeth and jaws, from a further series of rabbits.

Series 3 (Rabbits)

Operative procedure. About 1 in. of the right cervical sympathetic cord, between the middle and superior cervical ganglia, was removed from each of five rabbits; holes for measuring growth rate were again drilled in the lower incisor teeth, as previously described. The animals received the same diets as those in series 2.

Results. The effects of these operations are shown in Text-figs. 4, 8, 9 and 10. During the first 19 days after nerve resection, the average rate of growth of the lower incisor teeth of animals 19–23 was approximately: on the operated side, 0·28 mm. per day; on the unoperated side, 0·23 mm. per day; thus demonstrating that an increased rate of growth may also follow excision of part of the cervical sympathetic.

Probably, therefore, the effects attributed in series 1 and 2, to removal of the inferior dental nerve, were in reality due to injury to vaso-constrictor fibres. As in series 2, no periodontal changes were observed, but again opaque patches on both lower incisors were noted, and histological preparations demonstrated a larger blood content of the teeth of the operated side.

Discussion

(*a*) *Vitamin A deficiency* (*dogs*). M. Mellanby [1930], from experiments on dogs, concluded that vitamin A was primarily concerned with the development of the soft periodontal structures, while vitamin D

controlled that of the calcified tissues. Retarded tooth eruption, incisor irregularity, and the supervention of periodontal disease were considered to result from a combined deficiency of both these fat-soluble vitamins.

E. Mellanby [1926 *a*] found that in fat-soluble vitamin deficiency, susceptibility to infection was not related to the calcification of the bones, indicating that the former was due to deficiency of vitamin A rather than of D. Goldblatt and Benischek [1927] repeated the work of Mori [1922] and Wolbach and Howe [1925] on the dietary factor responsible for epithelial hyperplasia and metaplasia, but gave vitamin D in the form of irradiated cotton-seed oil, so that the diet was deficient only in vitamin A. The epithelial changes, as well as infective lesions also found, such as keratomalacia, broncho-pneumonia and enteritis, could now be definitely ascribed to vitamin A deficiency. As a result of further work on this problem, Green and E. Mellanby [1928, 1930] gave the name "anti-infective vitamin" to vitamin A, and later E. Mellanby [1933, 1934] showed that degeneration of sensory nerves, peripheral as well as central, resulted from lack of vitamin A.

In the experiments described here all of the dogs received vitamin D, the variable factor being vitamin A. Deficiency of the latter resulted not only in epithelial hyperplasia, degeneration of sensory nerves, and a somewhat increased deposition of cervical tartar, but also in retarded tooth eruption, irregularities in position of the lower incisor teeth, imperfectly formed tooth roots with a tendency to apical hypercementosis, ill-defined laminæ duræ and changes in the alveolar bone in some regions (Pls. I and II). It is of interest here to note that in some cases of chronic periodontal disease in man it is not uncommon to find rounded and stunted tooth roots similar in radiographic appearance to those observed in experimental vitamin A deficiency.

Although it is true that a combined deficiency of vitamin D and vitamin A accentuates some, but not all, of the abnormalities in the calcified structures and still further retards the eruption of the teeth, the results described here indicate that vitamin A, in addition to its influence upon epithelia and sensory nerves, may also play a part in the development of some of the calcified tissues of the body. In some unpublished experiments on rats the author has observed that vitamin A deficiency may be associated with degeneration of the ameloblasts and odontoblasts of their persistently growing incisors. In such A-deficient animals the characteristic enamel pigmentation was lost, while the dentine was poorly calcified and contained many interglobular spaces, conditions reminiscent of vitamin D deficiency, although the animals

were given ample amounts of vitamin D, calcium and phosphorus. The rat experiments confirm to some extent the findings of Wolbach and Howe [1933], who maintained that vitamin A played an extremely important part in the calcification of the teeth of rats. But the experiments of these investigators were not decisive, since their A-deficient rations were also deficient in vitamin D, while in their adequate diets vitamin A was given in the form of butter, a variable source of vitamin A which contains an appreciable amount of vitamin D.

It is usually considered that vitamin D controls calcification by mobilizing the mineral salts of the blood. It seems possible that vitamin A may affect calcification by its influence upon the morphology and consequent activity of the lime-secreting cells, even when vitamin D and mineral salts are available. If this is so, its absence may contribute to the development of periodontal disease and even dental caries by causing defects of tooth structure as well as by increased susceptibility to infection. It is hoped that the experimental production of such diseases, preliminary reports of which have already been published [King, 1935 *a*, *b*], may shed further light on this problem.

By means of a histological examination of human post-mortem material Wilkinson [1935] showed that degenerated cells of the subgingival epithelium may become calcified before the latter becomes separated from the tooth; later, when separation does occur, he claims that the calcified cells are left adhering to the tooth as calculus; the formation of the latter being similar to calcification in cartilage as described by Robison [1923]. In support of this theory Wilkinson cites the experiments of Smith [1930] and Adamson [1929], as demonstrating that desquamated cells of the gingival epithelia liberate a phosphatase, which, by setting phosphates free in the presence of calcium ions, allows calcium phosphate to be deposited in the dead cells. Wilkinson believes that calculus may thus be produced, resulting in the inflammatory complications so often encountered in human periodontal disease. Certainly epithelial degeneration, calculus formation, and gingivitis are common sequelæ to experimental vitamin A deficiency, so that since these conditions are also found in the human subject, whatever be the exact role of vitamin A in this syndrome, it may be inferred that lack of this vitamin, especially during the developmental period, is an important predisposing factor in the ætiology of periodontal disease.

(*b*) *Experimental nerve lesions* (*dogs and rabbits*). The relative uniformity in results of the experiments in series 2 and 3 (rabbits) indicates

that the effects produced by resection of the inferior dental nerve in both dogs and rabbits were caused by the unintentional severing of vaso-constrictor fibres in very close association with, and probably within the actual sheath of, the sensory nerve. It is probable, therefore, that in both species the temporarily increased rate of growth of the teeth peripheral to the lesion was due to an increase in the volume of blood available. Gilding [1932] showed that the distribution of the vaso-constrictor nerves is strictly unilateral, stopping abruptly at the mid-line, in spite of the fact that the blood vessels of one side anastomose freely with those of the other. The sensory distribution of the inferior dental nerve, on the other hand, frequently extends for some little distance across the mid-line. The growth changes here reported were confined to the operated side.

The temporary nature of such changes suggests that the walls of the affected blood vessels soon recover the greater part, at least, of their normal tone, and this may perhaps be why no histological alterations in the dental tissues were found by the methods described. It is true that microscopic preparations of the rabbits' lower incisor teeth after death showed more blood in the tooth pulp of the operated side, but this may be merely because the normally innervated vessels on the other side were constricted in the asphyxia induced by the chloroform used for killing the animals. In this respect the animals in which the inferior dental nerve was excised were like those in which the sympathetic was cut, so that it is probable that the constrictor nerves had been severed in all three series of animals and that the growth changes observed were due to this.

Leist [1927] described an increased rate of growth of the teeth of five out of nine guinea-pigs after cauterizing the sympathetic fibres around the carotid artery with 7 p.c. phenol; he also noted similar but less marked changes following such treatment of the internal maxillary and inferior dental arteries of a few dogs. In the cervical sympathetic of the dog there are dilator as well as constrictor fibres [Dastre and Morat, 1884], but not in the rabbit or cat [Feldberg and Schiff, 1926]. As the constrictors predominate over the dilators in the dog's splanchnic nerve [Dale, 1913], this may also be expected in its cervical sympathetic.

Although resection of the inferior dental nerve, as previously described, has failed to cause lesions in the teeth or periodontal tissues, it is possible that, by destruction of the whole sensory supply to one side of one or both jaws and by preventing nerve regeneration for a sufficiently

long period, pathological changes similar to those seen in vitamin A deficiency may be produced. Experiments along these lines are now in progress; in some, the sensory roots of the fifth nerve are resected, while in others the Gasserian ganglion from which they originate is itself removed.

The results of the present nerve operations may have some bearing on the vexed problem of tooth eruption, for they show that, in addition to vitamins A and D, the blood supply and condition of the vascular nerves play a part in the development and growth of the teeth. Gottlieb [1927] claims that changes in the rate of growth of the teeth are of outstanding importance. He believes that eruption continues slowly after the teeth have come into occlusion, and that any conditions which disturb the synchronism between attrition of the tooth crowns and "postclinical" eruption accelerate the downgrowth of the subgingival epithelium and the development of periodontal disease. If the misalignment of the right lower incisors following the operation in the adult dog which received vitamin A was due to changes in the blood supply, it seems possible that such changes, resulting in abnormal movements of the teeth even after complete clinical eruption, may in turn give rise to changes in their supporting structures. The fact that in this dog the periodontal tissues appeared free from defects and there was no downgrowth of subgingival epithelium along the root or absorption of alveolar bone, does not seem to support Gottlieb's hypothesis, although more experiments on adult animals are necessary. Movements of the teeth after clinical eruption are, of course, quite commonly seen in the human mouth, associated with trauma, pressure from other teeth, or extraction of adjacent or opposing teeth.

Summary

In dogs receiving vitamin A-deficient diets:

(1) eruption of teeth was delayed;

(2) hypercementosis occurred and the laminæ duræ and bone of the tooth sockets were malformed;

(3) the alignment of the incisor teeth was irregular; in addition to the periodontal and nervous defects described in previous papers.

In animals (dogs and rabbits) in which the inferior dental nerve was resected on one side:

(1) the eruption in dogs, and in rabbits the growth of teeth was accelerated;

(2) the alignment of the incisor teeth was irregular; but no defects in the dental or periodontal tissues were found.

As similar changes were observed on resection of the cervical sympathetic, the effects of severing the inferior dental nerve are regarded as due to damage to vasomotor fibres in it.

The expenses of this investigation have been defrayed by the Medical Research Council, to whom my thanks are due. I am also indebted to Mrs M. Mellanby and Prof. G. A. Clark for their valuable advice and criticism, and to Miss I. Jopling for clerical assistance.

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Fig. 1. Dog *a*.

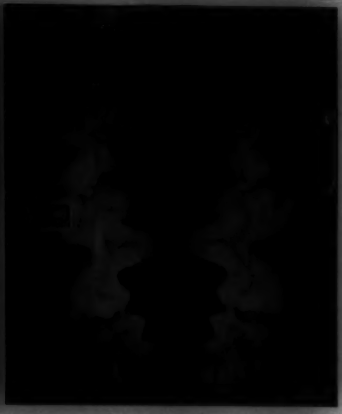

Fig. 2. Dog *b*.

Fig. 3. Dog *c*.

Fig. 4. Dog *g*.

To face p. 76

Fig. 1. Dog *a*.

Fig. 2. Dog *b*.

Fig. 3. Dog *c*.

Fig. 4. Dog *g*.

Fig. 5. Dog *a*.

Fig. 6. Dog *b*.

Fig. 7. Dog *g*.

EXPLANATION OF PLATES I AND II

Plate I

Radiographs to illustrate the effects of vitamin A deficiency and of resection of the right inferior dental nerve upon the lower jaw and teeth of dogs.

Fig. 1. Dog *a*. Age at operation, 22 months. Postoperative period, 12 months. Diet contained liberal supplies of vitamin A. The appearance of the teeth and bone of both sides is normal.

Fig. 2. Dog *b*. Age at operation, 11 months. Postoperative period, 11 months. Diet was deficient in vitamin A.

Note. Defective tooth roots, ill-defined laminæ duræ and, surrounding these, the bone trabeculæ are more radio-opaque on both sides.

Fig. 3. Dog *c*. Age at operation, 2 months. Postoperative period, 6 months. Diet included liberal amounts of vitamin A. The teeth and bone of both sides appear normal.

Fig. 4. Dog *g*. Age at operation, 2 months. Postoperative period, 5 months. Diet was deficient in vitamin A but calcium carbonate was added.

Note. On both operated and unoperated sides the tooth roots are defectively formed, the laminæ duræ are not well-defined and the surrounding bone trabeculæ are more radio-opaque than normal; the lower margin of the jaw of this animal was injured after death.

O, site of operation. *A*, *B*, *C*, areas from which the photomicrographs shown in Pl. II were prepared.

Plate II

Photographs and photomicrographs to show the effects of vitamin A deficiency and of resection of the right inferior dental nerve of the dogs referred to in Pl. I.

Fig. 1. Dog *a*, operated when 22 months old. Diet contained liberal amount of vitamin A.

Note. Irregular alignment of the lower front teeth of the operated side.

Fig. 2. Dog *b*, operated when 11 months old. Diet was deficient in vitamin A.

Note. Irregular alignment of lower incisors of both operated and unoperated sides; the canines were removed.

Fig. 3. Dog *c*, operated when 2 months old. Diet included liberal supplies of vitamins A and D.

Note. Irregularity of lower incisors of the operated side.

Fig. 4. Dog *g*, operated when 2 months old. Diet was deficient in vitamin A but calcium carbonate was added.

Note. Bilateral irregularity of lower incisors.

O, operated side.

Fig. 5. Ground section of area A, Pl. I, dog *a*.

Note. Normal appearance of apical and peri-apical tissues; section is not quite central, but, when considered in conjunction with the radiograph (Pl. I, fig. 1), the tissues are seen to be free from obvious defects.

Fig. 6. Ground section of area B, Pl. I, dog *b*.

Note. Invasion and replacement of pulp and dentine by cementum.

Fig. 7. Ground section of area C, Pl. I, dog *g*.

Note. Invasion of pulp and dentine by cementum.

612.748:595.384

THE DOUBLE MOTOR INNERVATION OF THE ADDUCTOR MUSCLE IN THE CLAW OF THE CRAYFISH[1]

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(Received May 11, 1936)

I. Introduction

Keith Lucas [1917] discovered that the adductor muscle of a crustacean, *Astacus fluviatilis*, can exhibit two kinds of contraction, a quick one and a much slower one of a more tonic character, which he distinguished as twitch and slow contraction. These two contractions have later been observed by several authors [ten Cate, 1927; Blaschko, *et al.* 1931; Wiersma, 1933]. Lucas compared the strength-duration curves in *Astacus* for both contractions and concluded that they are caused by the stimulation of two different elements in the nerve. Wiersma stimulated the nerve of several crustaceans with brief direct currents and found that the height of the twitch grew by steps when the duration or potential of the stimulus was increased continuously. With longer lasting but weaker currents pure slow contractions were obtained in *Astacus*. The slow contraction does not show a step-like increase, but increases continuously with the strength or duration of the stimulus. As Wiersma found that the action current of the first step of the twitch, which showed only one spike, became double topped when the second step was reached, he assumed that short direct-current stimulation can evoke more than one impulse in the nerve, and that the transition from one step to the next indicates that an extra impulse has been set up in the nerve. As the chronaxie of the first step was long ($1{\cdot}4\sigma$ in *Homarus* and *Astacus*), he assumed that even the first step is caused by more than one impulse. This assumption made the following hypothesis possible: the twitch and the slow contraction are caused by an impulse train in the same nerve fibres, and it will depend on the frequency of these impulses

[1] A preliminary communication of part of the results appeared in *Proc. Nat. Acad. Sci.*, Wash., 22, 190 (1936).

whether a twitch or a slow contraction is set up in the muscle, the higher frequencies giving the twitch, the lower ones the slow contraction. This view was supported by later experiments [Wiersma & van Harreveld, 1934*a*, *b*] on the abductor of the claw of *Eupagurus bernhardus*, which also gives a twitch and a slow contraction (there are, however, differences between the twitch in *Eupagurus* and in *Astacus*). In this same muscle we could demonstrate peripheral inhibition. As this muscle seems to have only two nerve fibres and one of them has to be reserved for the peripheral inhibition we concluded that the other fibre must evoke the twitch as well as the slow contraction. However, experiments on *Cambarus clarkii*[1] and on *Astacus trowbridgii*[1] showed that Wiersma's hypothesis does not hold good at least for the adductor of these crustaceans. In the following pages we shall describe experiments which make it clear that in these animals the twitch and the slow contraction are caused by the stimulation of different fibres in the nerve.

II. The twitch contraction of the adductor

A. *The first step*

We have measured the chronaxie and the strength-duration curve for *Cambarus clarkii* and for *Astacus trowbridgii*. For this we used rectilinear direct-current stimulation, the voltage being regulated by a potentiometer. In most of the experiments the chronaxiemeter was a Lucas contact breaker, in some a Lucas pendulum. As currents of longer duration can evoke more than one nerve impulse, this method of measuring chronaxie is to be preferred to the use of condenser discharges, as with rectilinear currents the strength stays more constant for the first and for the following impulses. Silver chloride electrodes were applied through holes in the meropodite, or to the nerve dissected out of the meropodite. The electrodes were normally about 7 mm. apart. The room temperature varied from 18 to 25°. This method is essentially the same as that used previously [Wiersma, 1933].

Cambarus. The smallest twitch contraction that can be obtained, isotonically, without load, in a fresh preparation is fairly large and causes complete closure of the claw. Isometrically the tension developed is of the order of 30 g., when the lever is directly attached to the tendon. The contraction is the same for different strengths of stimulation, and does not

[1] *Cambarus clarkii* Girard has been identified as such for us by Dr W. L. Schmitt of the United States National Museum, Washington, D.C.; *Astacus trowbridgii* by Dr H. W. Clark of the California Academy of Sciences, San Francisco. We are greatly indebted for these identifications.

increase with longer stimuli unless a second step is set up. The height of contraction diminishes, of course, with fatigue, so that the "all" of the all-or-none becomes less. Table Ia shows a number of chronaxie values for the first step of the twitch; the average value being 0·3σ. If we compare this value with that of a frog's nerve-muscle preparation (sciatic

TABLE I. Chronaxie (pseudo-chronaxie) of some steps of the contraction of the adductor of:

A. *Cambarus clarkii*

Temperature °C.	First step σ	Second step σ	Third step σ	Distance of electrodes mm.
20	0·2	—	—	—
20	0·15	—	—	—
24·5	0·4	4·1	—	—
—	0·25	—	—	—
—	0·25	—	—	—
20	0·4	5·0	—	—
—	0·3	—	—	—
21	0·15	—	—	8
22	0·25	—	—	9
20	0·25	—	—	5
20	0·4	4·5	—	10
21	0·5	4·0	8·3	11
20	0·25	—	—	10
—	0·4	—	—	—
—	0·25	—	—	10

B. *Astacus trowbridgii*

Temperature °C.	First step σ	Second step σ	Distance of electrodes mm.
—	0·45	—	—
—	0·3	—	—
—	0·25	—	—
21	0·15	—	7
22	0·5	—	8
21	0·25	—	—
20	0·35	—	7
21	0·35	—	10
22	0·15	—	7·5
22	0·45	4·0	8

and gastrocnemius of *Rana esculenta*) which is about 0·3σ, we must conclude that this chronaxie value is too short to be a pseudo-chronaxie, in other words a single nerve impulse will evoke a contraction. In *Astacus fluviatilis* the chronaxie of the first step of the twitch measured in the same way is much longer: 1·2–1·4σ [Lucas, 1917, Wiersma, 1933]. The strength-duration curve for *Cambarus* was plotted and showed a normal shape (see Fig. 1). With single induction shocks as stimulus the same all-or-none relation of the first step of the twitch can be demonstrated.

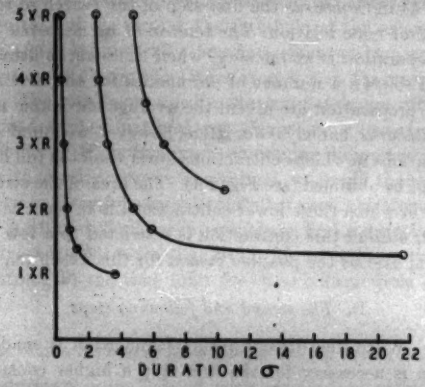

Fig. 1. Strength-duration curve of three steps of the twitch of the adductor muscle of *Cambarus clarkii*. Units on the ordinate are the rheobase of the first step of the twitch.

Fig. 2A.

Fig. 2B.

Fig. 2A. *Astacus trowbridgii*. Intermediate contractions of the adductor muscle, lower than the first step of the twitch. Stimuli of a duration of 0·35 and 0·55σ evoke the intermediate contraction, those between 0·7 and 3·6σ give the full first step. Isotonic, with load. Stimulating electrodes in meropodite, distance 10 mm.

Fig. 2B. *Cambarus clarkii*. Three steps of the twitch of the adductor. The first step is elicited by a duration of the stimulus between 0·2 and 2·2σ, the second step between 2·3 and 4·5σ, above this a third step can be seen. Stimulating potential 3·2 volts. Distance between the electrodes 8 mm. Isometric.

Astacus. As in *Cambarus* the first step of the twitch of the adductor showed an all-or-none relation. The tension of an isometric contraction in a fresh preparation is about 60 g. when the lever is attached to the tendon. In Table Ib a number of chronaxies for the first step of this nerve-muscle preparation are given, the average chronaxie is about the same as in *Cambarus*, namely 0·3σ. Here, however, we found a few exceptional preparations in which contractions lower than the full height of the first step could be obtained (see Fig. 2A). The area of the strength-duration diagram in which these lower contractions take place is very small. With stronger stimuli this contraction is restricted to a few tenths of a sigma. We will discuss the possible reason for this exception later.

B. *The second and following steps*

Cambarus. When the direct-current stimulation is made longer or stronger than is necessary for the first step a higher contraction may occur (see Fig. 2B). The growth in height is, however, discontinuous, and steps can be distinguished each having its own constant height.[1] The number of steps which can be readily detected by their differences in height is, however, limited, as these differences become smaller for higher steps and are then of the same order as the changes in height caused by fatigue and recovery. It is thus possible to measure the pseudo-chronaxie for a number of these steps. The pseudo-chronaxies for these steps are fairly constant, but the relation between the rheobase of the first step and the second one is very variable, even in one preparation. Normally the rheobase of the second step increases with time, and may become so high during the experiment that it becomes practically impossible to elicit this and the higher steps, the rheobase of the first step remaining about the same as before. In many preparations this condition seems to prevail at the very start of the experiment, so these preparations do not give any higher steps. For this reason we were able to determine the pseudo-chronaxies and the strength-duration curves of the higher steps in only a few cases. The pseudo-chronaxie of the second step found in these was about 4·5σ, of the third step it was about 8·5σ. Fig. 1 shows the strength-duration curve of several steps of one preparation. As will be seen there is a marked relation between the durations of threshold currents for the different steps. At least for higher voltages the increase of the

[1] Excluding fatigue, the heights of the higher steps are constant for currents of the same voltage but differing in length. Also all currents above the approximate pseudo-chronaxie voltage give contractions of the same height; below this voltage differences in height with different strengths of current may be observed in one step, but they are not pronounced.

stimulation time for a certain voltage needed to change the threshold of the first into that of the second, the threshold of the second into that of the third step, etc., is practically the same, a fact to which we have already drawn attention in *Eupagurus* [Wiersma & van Harreveld, 1935].

Astacus. The results obtained in this animal do not differ essentially from those we described for *Cambarus*. The pseudo-chronaxie of the second step was about 4σ. Here, too, it was sometimes difficult to get the higher steps. As with the first step in this animal, we found that some preparations do show contractions intermediate between two steps; with higher voltages it is often difficult or impossible to find a duration of stimulus that will give such a contraction. With lower voltages (below pseudo-chronaxie strength) the time limit for these contractions may be fairly long.

C. *The action current of the adductor during twitch contractions*

Action currents were led off with electrodes, which consisted of U-tubes filled with physiological solution, from which cotton plugs soaked in this solution lead to two holes in the shell. Usually one lead was

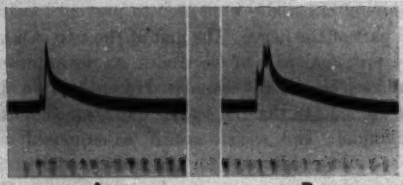

Fig. 3. *Cambarus.* Action currents of the first and the second step of the twitch contraction Nerve stimulated in the meropodite, the nerve bundle containing the fibre for the abductor muscle transected. The stream escape shows the duration of the stimuli. Stimulating potential 0·7 volt. Time 1/50 sec. A. Duration of the stimulus $0{\cdot}54\sigma$. B. Duration of the stimulus $9{\cdot}9\sigma$.

applied to the unmovable tip of the claw, the other through a hole made in the shell covering the middle of the muscle. In the U-tubes silver-silver chloride spirals dipped, and these were connected with the amplifier of a Matthews' oscillograph. To diminish escape of the stimulating current the nerve was in a number of cases prepared and put directly on electrodes instead of applying the electrodes through holes in the shell. With increase of the strength of the stimulus there appears at the same time as the first step of the twitch an action current which reaches

its full height immediately. The action currents of the second step are difficult to obtain. This is because preparations in which the nerve has been dissected out will respond to any strength of stimulus with the first step only. If the nerve is left in the meropodite higher steps may be elicited, but usually the stimulus has to be then so strong that the record of the action current is quite distorted by the stimulus escape. The pictures obtained showed clearly that the action current of the second step consists of two spikes, which may be more or less separated. In Fig. 3 we give an example of the action currents of a first and a second step of one preparation.

D. *Summation, stimulating with two single induction shocks*

Two induction shocks, applied by two pairs of electrodes, were obtained from two induction coils, which were at an angle of 90°. The primary circuits of the induction coils were switched off by two contacts of a Lucas contact breaker. Care was taken that the direction of the two shocks was the same and that both were descending. The separation of the two sets of electrodes was usually about 3 mm., the electrodes of each pair being 2 mm. apart, making the distance between the two cathodes 5 mm. Two pairs of electrodes were used to prevent local summation in the stimulated part of the nerve. The first of the two stimuli was sent in by way of the proximal pair of electrodes, so no impulse had to pass a place that had been electrically stimulated recently. Two kinds of experiments were performed. In the first set the induction shocks applied were both just subliminal. This was to see, if, as supposed in Wiersma's hypothesis, a stimulus subliminal for the muscle contraction or for the action current is able to set up an impulse in the nerve. With two subliminal stimuli, if each of them sets up one impulse as supposed, a contraction of the muscle would be expected. In view of our results on the chronaxie of the first step, this seemed highly unlikely. The results confirmed this last view. Summation of two subliminal stimuli never resulted in a muscle contraction or an action current. When a contraction resulted, it could always be shown that one of the stimuli had passed the threshold and caused a contraction when given alone. This confirms the conclusion that the first step of the twitch is caused by one impulse in the nerve only. Thus the assumption that the twitch and the slow contraction are caused by an impulse train in the same nerve fibre has to be discarded and the problem of the double motor innervation again becomes of importance.

In a second set of experiments each of the two stimuli was made so strong that it was somewhat above the threshold of the first step of the

twitch. Lucas [1917] carried out similar experiments with *Astacus* (*fluviatilis*), but only with preparations in which the twitch caused by a single induction shock had disappeared through fatigue. He plotted the height of contraction against the interval of time between the two stimuli. In about one-third of the cases he found a slow increase in height with longer time intervals, with a maximum between 5 and 15σ. In the other cases this slow increase was preceded by an earlier maximum between

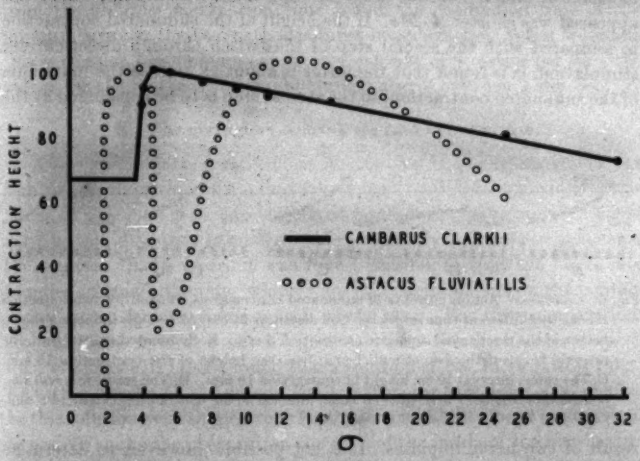

Fig. 4. Curve showing the relation between the height of a summated contraction and the time interval between the two summating stimuli. Comparison between the relation that we found for *Cambarus clarkii* and the relation as given by Lucas [1917] for *Astacus fluviatilis*. In both cases the maximum summated height was taken as 100.

2 and 5σ. According to him the early maximum is due to the twitch, the second one to the slow contraction. In Fig. 4 we reproduce one of his curves. Our results were totally different from his, as we found the early maximum only (see Fig. 4). While Lucas used stimuli many times the strength of the threshold stimulus, which certainly can evoke also a slow contraction [see Wiersma, 1933], and as our stimuli were always so weak as to cause a quick contraction only, the absence of the second maximum is readily accounted for. Compared with his early maximum our maximum showed marked differences. Instead of dropping quickly after reaching its maximum there is a continuous slow decrease in height

when the time interval is increased. We found as he did, that the maximum is not reached at once, but that it is preceded by an increase in height which, however, is much steeper than in the curve of Lucas. The maximum lies at about 4·5σ. Our experience was that preparations in which one stimulus still gave a contraction showed summation over a long range of time intervals, 4σ to more than 40σ; preparations in which a contraction no longer resulted from a single shock would also show a mechanical effect, but the range of time intervals through which this appeared was shorter, 4–20σ. If the height of the summated contraction is compared with the second step of the twitch through direct-current stimulation, it is found that the latter is about as high as the maximum of the summated contraction, so the second step is to be considered as the

Fig. 5. *Cambarus*. Action currents of summated contractions, evoked by two induction shocks with different time intervals. Coil distances 25 cm. A. A single induction shock, height of the mechanical isometric contraction 6 mm. B. Summated contraction, the interval between the two stimuli being 5·4σ, the height of the contraction 19 mm. C. The same, interval 10·8σ, height of contraction 18 mm. D. The same, interval 18σ, contraction height 17 mm. E. The same, interval 30·5σ, contraction height 13·5 mm.

result of two nerve impulses. It is not possible, however, to determine more exactly where the second step of the twitch is located on the curve of the summation; so we cannot know from this the exact interval between the two nerve impulses in direct-current stimulation. That the height of the second step of the twitch can be slightly changed with different voltages must be because of differences in the time between the two impulses set up in each case in the nerve (see footnote, p. 82).

The action current showed two definite spikes when the time interval between the two stimuli was large; these spikes came closer and closer together as the time interval was diminished (see Fig. 5). The action currents of the contraction evoked by two induction shocks with the shortest time interval that still gives summation became more or less fused. It is remarkable that the summated action current is not higher but only longer than the single one, contrary to those of other crustaceans, e.g. *Eupagurus bernhardus* [Wiersma & van Harreveld, 1935].

Though the methods of leading off are the same in both cases we do not yet know if this difference is essential, as the pennated structure of the muscle may complicate the action current. The results obtained with concentric needle electrodes did not throw further light on this problem, as the action currents were polyphasic in these experiments.

The least interval between the two stimuli that still gave summation must depend on two factors—first the time taken for the first impulse to reach the second cathode, and second the real interval at this place; so from the shortest summation time found the conduction time of the impulse between the two cathodes must be subtracted. When we make this correction (assuming a conduction velocity of 8 metres per sec.) the values for the real least interval obtained are all between 2 and 3σ.

E. *The nerve fibre causing the twitch contraction*

The experiments described above lead to the conclusion that the twitch and the slow contraction are evoked by different kinds of nerve fibres. We then tried to isolate these fibres from the nerve bundle. A first step in this direction has been made previously in marine crustaceans [Wiersma, 1933], where it was found possible to split the nerve into bundles of small diameter which were still excitable and might contain motor axons for one single muscle of the claw. For these preparations sea water was used as a physiological solution. For fresh-water crayfish van Harreveld [1936] found that a solution of NaCl 12 g., $CaCl_2$ 1·5 g., KCl 0·4 g., $NaHCO_3$ 0·2 g., and $MgCl_2$ 0·25 g. per litre gives good results. In this solution even single axons kept their excitability for at least several hours. To make the preparation the shell of the inside of the meropodite is removed, the tendon of the flexor muscle of the carpopodite is cut and the muscle removed. It is best also to remove the extensor of the carpopodite, so that the nerve is free of surrounding tissue along its course in the meropodite. The propodite of the claw is fixed and the whole preparation submerged in the fluid in a Petri dish. Under a binocular microscope the nerve is then divided with a sharp-pointed needle into bundles. These bundles are to a certain degree already preformed. After some practice it is possible to predict in which of these the axons looked for will be situated. This is valuable, as it reduces considerably the number of times that this bundle has to be stimulated, and so unnecessary fatigue is avoided. The nerve bundles are tested with short faradic stimulations, and those not containing the motor fibres looked for discarded. In the preliminary experiments the movements of the stimulation electrodes was rather coarse, and this made it impossible to prepare nerve bundles beyond the

division in which all the motor fibres for only one muscle were present, as thinner bundles were always damaged during stimulation. By using thin platinum wire electrodes moved by a micromanipulator this difficulty was overcome and single motor fibres could be isolated. The last part of the preparation often takes considerable time, as the fibres in one bundle stick quite firmly together and are also sometimes twisted. It is in general possible to free a long part of the fibre, though this was usually not necessary for experimental purposes. The twitch during faradic stimulation can be easily distinguished by sight from the slow contraction. In all the preparations of *Cambarus* made up till now (more than 100) we have found in each only one axon causing the twitch of the whole adductor muscle. This fibre is among the thickest of the nerve: the diameter measured was between 40 and 90μ. As might be expected from the experiments with the whole nerve, this fibre sets up a twitch contraction when stimulated with single induction shocks. Stimulation with long-lasting direct currents seldom gave a higher step of the twitch. That the chronaxie of the preparation does not change when isolation was carried

TABLE II. Strength-duration measurements of the adductor of *Cambarus clarkii*. First step of the twitch. In A silver-silver chloride electrodes were used, in B and C U-tubes containing physiological solution, connected with a cotton wick to the preparation and in which silver chloride coated spirals dipped. Electrode distance 10 mm.

Rheobase	Voltage	Time in σ
A. Right claw, nerve not prepared.		
1 ×	0·52	8·0
2 ×	1·04	0·40
3 ×	1·56	0·30
5 ×	2·60	0·12
2 ×	1·04	0·40
B_1. Right claw, nerve prepared		
1 ×	0·6	8·0
2 ×	1·2	0·40
3 ×	1·8	0·24
5 ×	3·0	0·20
1 ×	0·75	8·0
2 ×	1·5	0·40
5 ×	3·75	0·16
B_2. Left claw, nerve prepared.		
1 ×	0·23	8·0
2 ×	0·46	0·40
3 ×	0·70	0·28
5 ×	1·15	0·16
C. Left claw, small nerve bundle prepared.		
1 ×	0·18	8·0
2 ×	0·36	0·40
3 ×	0·54	0·24
5 ×	0·90	0·16

out is shown by the following experiment. The chronaxie was first measured with chloride plated silver wire electrodes pushed into the meropodite; after this the nerve was prepared and the chronaxie was determined again using electrodes consisting of U-shaped tubes filled with physiological solution in which the coated silver spiral dipped. Then a fine nerve bundle containing the motor fibre for the twitch contraction was prepared and the chronaxie determined with the same electrodes. In every instance the chronaxie was the same, namely 0·4σ. The results of this experiment are collected in Table II.

In *Astacus trowbridgii* the same single fibre for the twitch contraction can be isolated; it is somewhat easier to prepare in this animal.

F. *The action current of the nerve fibre evoking the twitch*

It is possible to lead off from the single motor fibre for the twitch contraction. For the stimulating as well as for the leading off electrodes the above-mentioned fluid electrodes were used. The fibre was prepared for about 5 mm. distance in the middle of the available part of the nerve in the way mentioned above. In such a preparation the nerve in the carpopodite as well as that in the meropodite was used. To prevent drying out, the single-fibre part of the nerve stayed in the physiological solution, the undivided parts of the nerve on both sides of the single fibre were lifted out and placed on the electrodes. The action current obtained in this way consisted of one spike only, which is in accordance with the difficulty found in setting up higher steps of the twitch on direct-current stimulation when the electrodes are applied directly to the nerve. Like the muscle-action current the electrical phenomenon of this nerve fibre showed, as far as we could determine, the all-or-none relation.

III. The slow contraction of the adductor

A. *The mechanogram*

While in *Astacus fluviatilis* a slow contraction could usually be obtained [Wiersma, 1933] by stimulating the nerve with long-lasting currents below the rheobase of the twitch, in most cases no effect at all results from such a stimulus in preparations of *Cambarus* and *Astacus trowbridgii*. In the few preparations in which the slow contraction could be obtained separately in this way, the contraction grew continuously with the increase of the strength of the stimulus, confirming the results obtained before. Stronger, long-lasting stimuli usually gave a twitch

followed by a slow contraction. Another way to obtain pure slow contractions, without preparing and transecting the twitch fibre, was to use a weak faradic stimulation, though with this stimulus the slow contraction was often preceded by a twitch. In a preparation in which the twitch fibre was discarded, the pseudo-chronaxie of the slow contraction could be

Fig. 6. *Astacus troubridgii*. Fibres for the slow and for the twitch contraction were prepared and stimulated faradically. Coil distance for both 25 cm. Time in seconds. Isometric, the lines parallel to the zero line indicate the tension in grams. A. Faradic stimulation of the twitch fibre. B. Faradic stimulation of the fibre for the slow contraction. C. Stimulation of the fibre for the twitch contraction again.

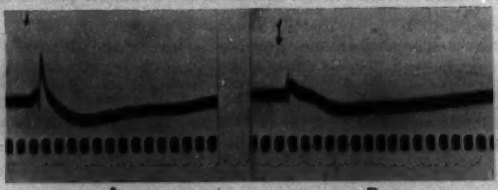

Fig. 7. *Cambarus*. Muscle action currents of the adductor muscle during long direct-current stimulation of the nerve with two electrodes pushed into the meropodite. The bundle containing the motor fibre for the abductor muscle was transected. Only the "make" of the current is shown. Time 1/50 sec. A. Stimulating potential 1 volt. The record shows the action current of a twitch contraction, followed by the small spikes of the action current of a slow contraction. B. Stimulating current 0·2 volt. Action current of the slow contraction only.

measured. If the first visible contraction was taken as an indicator both for rheobase and chronaxie, the value found was in the region of 20σ, which is in agreement with the pseudo-chronaxie of the slow contraction in *Astacus fluviatilis*. It is interesting to compare in the same preparation in which the twitch fibre had been separated, the fast and the slow contraction during faradic stimulation (see Fig. 6). Using a frequency of about 40 per sec., the fast contraction reaches its maximal tension when not fatigued in about 1·5 sec., under the same conditions the slow one

takes about 30 sec. to reach its maximum. The tension developed in the slow contraction was almost as much as that during the faradic stimulation of the twitch fibre. In a certain experiment the slow developed a maximum tension of about 500 g., the fast somewhat more, namely 650 g. When the faradic stimulation is repeated, another striking difference between the fast and the slow contraction became visible. Whereas the top of the fast contraction is reached in the same time or somewhat later in the repeated contraction the slow contraction reaches its maximum earlier with each repetition. So in one case it was found that the top of this contraction was reached on the first stimulation after 30 sec., on the second after 20 sec., on the third after 14 sec. and on the fourth after 12 sec. In each case almost the same maximal tension was developed, so the early maximum in the later contraction cannot be due to fatigue. Through the fatigue the duration of the maximum tension is shorter. The fast contraction is much sooner exhausted than the slow one. After some alternative stimulations of the slow and fast fibre bundle, the fast contraction was almost completely exhausted; it developed in the above preparation only 50 g., while the slow contraction showed only a little fatigue, and still developed 450 g. Under our experimental conditions the slow contraction has a much faster recovery than the fast one. After the exhaustion of the fast contraction a rest of some minutes does not bring the reaction back, whereas after 80 sec. of stimulation of the bundle containing the slow fibre(s), at the end of which the tension had dropped to about 100 g., a few minutes' rest were sufficient to allow the muscle to recover so much that a contraction of the same tension (500 g.) as before was obtained. There are no large differences of the relaxation speed of the slow and the fast contraction. As might be expected the relaxation of both becomes slower after several stimulations.

B. *The muscle-action currents of the slow contraction*

In a few instances pure slow contractions could be obtained by stimulating the whole nerve with long-lasting direct current: a more certain method, however, was to remove the twitch fibre and stimulate the remaining part of the bundle. The action currents show, after a relatively long latent period, small deflections, which if the stimulus is continued grow in size and diminish in frequency. The frequency depends on the strength of the stimulus and can become as high as 200 per sec. at the beginning of the stimulation. As the crests may be quite separated from each other, the growth in height cannot be a summation of the potentials of different spikes, but every spike really develops a higher potential

than the preceding one. From the long latent period it is likely that the action current becomes visible only after some nerve impulses have already reached the muscle, though high amplification has been used for recording the action current. The contraction again becomes visible after several tops of the action currents have been set up. These data are in complete agreement with those found in *Astacus fluviatilis*.

When leading off action currents it was found that in one preparation under unchanging circumstances there exists a great difference in the height of the action current of the twitch and of the slow contraction. Even the largest deflexions at the end of a slow contraction do not develop a potential of more than one-tenth that of the unfatigued twitch. In another set of experiments the action currents of the muscle were led off during faradic stimulation of a nerve bundle which elicited the slow contraction

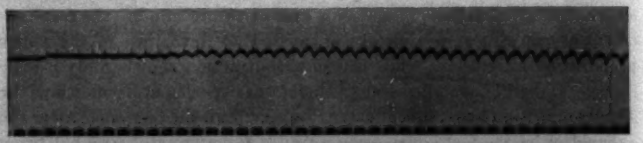

Fig. 8. *Cambarus*. Muscle action currents of the adductor muscle evoked by faradic stimulation of the fibre for the slow contraction. Coil distance 25 cm. Time 1/50 sec.

only. As we have seen the mechanical effect of such a stimulation may take as long as 30 sec. to reach its maximum. The action current, however, shows an increase in height during a much shorter period, so that the growth in the mechanical effect does not seem to be accompanied by a continuous growth of the electrical phenomena. Faradic instead of direct current stimulation shows even more clearly that the first nerve impulses do not evoke muscle action currents; on the first four to five stimuli no trace of an action current could be detected in a fresh preparation (see Fig. 8). After this the gradual increase took place. On cessation of a long faradic stimulation there were never any action currents after the one set up by the last induction shock, and no after discharge was observed.

C. *The motor fibre for the slow contraction*

After we had found that the twitch fibre could be obtained and stimulated separately from a bundle whose only effect was to give a slow contraction, we thought that further preparation of the slow axons

would be very hard, as we considered it likely that such axons would belong to the large group of very thin fibres which make up most of the nerve. To our surprise we found that the slow contraction also is evoked by the stimulation of one fibre only, and this axon too is amongst the largest fibres of the nerve. In *Cambarus* its diameter was between 25 and 50μ. The relation of the diameters of the fibres for the twitch and for the slow contraction can be seen from Table III; the ratio was about 1 : 1·5.

TABLE III. Diameter of slow and twitch axon

Preparation	Slow axon in μ	Twitch axon in μ	Ratio
A	33	45	1·37
B	51	74	1·44
C	42	60	1·43
D	30	50	1·66
E	45	60	1·34
F	25	40	1·60
G	40	57	1·42
H	42	90	2·14
I	36	60	1·91
J	36	48	1·33
Mean	38	58	1·5

So the adductor of the claw of *Cambarus*—and this holds true for *Astacus trowbridgii*—is innervated by two motor axons only, each of which has a different function. There is no doubt that in normal preparations of the species we used in these experiments there are never more than two motor axons for the adductor. A histological investigation of the motor innervation of the muscles in the cheliped is in progress and will be published later. With regard to the adductor muscle the result obtained was that it is innervated by only two thick axons.[1] This is in full confirmation of the extensive physiological tests in which it was found that no other fibres on stimulation gave any contraction in the adductor muscle but these two. This could be proven in all the preparations but those made from regenerated claws, in which often at least four fibres instead of two were found to cause a contraction when stimulated. Such preparations are difficult to work with, as the nerve fibres are often twisted, and isolation of a single fibre without damaging it is nearly impossible. In normal preparations the two fibres are usually close together, and the division of the two is in most cases the last step to be done to get single axons. Even with careful preparation and staining of the fibres with methylene blue, no branching of either the slow or the fast motor fibre could be observed during their course in the meropodite.

[1] In the same paper evidence will be given that this muscle usually lacks inhibitory fibres, though these are present in other muscles of the cheliped.

D. *The action current of the slow fibre*

The method used to obtain action currents of the slow fibre only was exactly the same as we described for the fast fibre. Long-lasting direct current was used as stimulus. In contrast with the effect on the fast fibre this stimulus usually sets up in the slow fibre a series of action potentials. Contrary to what we saw in the muscle action current during the slow contraction these spikes are all of the same height. This makes it likely that the all-or-none relation also holds true for the slow motor fibre. The short latent period of the first spike of the action current in the fibre confirms the view mentioned before, that the first nerve impulses

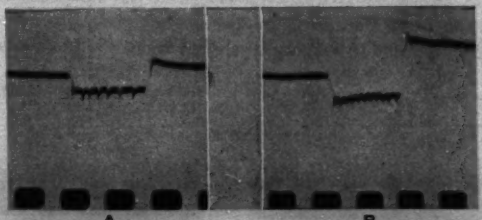

Fig. 9. *Cambarus*. Action currents of the axon evoking the slow contraction of the adductor muscle of the claw. The diameter of the fibre was 30 μ. Time 1/50 sec. A. Stimulating potential 0·34 volt. In this curve it can be easily seen that the frequency of the discharges at the beginning of the stimulation is higher than at the end. B. Stimulating potential 0·6 volt. Duration of stimulus was in both cases 34σ.

reaching the muscle do not set up detectable action currents there. Though the chronaxie of this nerve fibre has not yet been measured exactly, it seems to be short, and much shorter than the pseudo-chronaxie of the slow contraction. The impulses in this fibre are very much like those we found in the twitch nerve fibre. The frequency varies with the strength of the stimulus. For example, a stimulus of 0·34 volt for 34σ set up six impulses in the nerve; increasing the voltage to 0·60 volt gave ten impulses during that time (see Fig. 9). In another preparation direct currents lasting about 0·5 sec. were used. By this method the action currents always stopped before the stimulating current was broken. In Fig. 10 the results of these experiments are given. It is clear that the frequency always decreases as the stimulus continues, but the stronger the stimulus the higher the mean frequency [see Jasper & Monnier, 1933]. The frequencies observed in the nerve fibre support the

view that each nerve impulse sets up a peak in the electric response of the muscle during the slow contraction (excepting the first impulses to which the muscle does not give any electrical response), as both have the same frequency and in both the frequency of the action currents decreases in the same way during the stimulus.

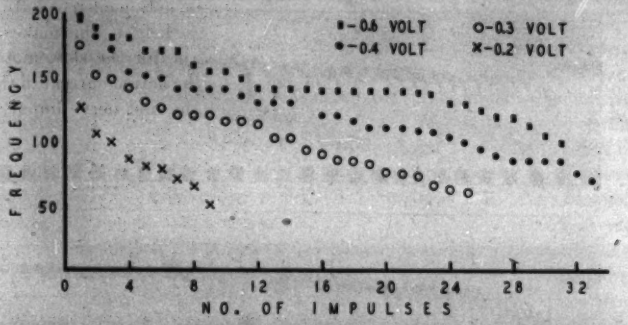

Fig. 10. Curve showing the relation between the frequency of a given impulse, in a train of impulses set up in the fibre for the slow contraction of the adductor muscle of *Cambarus*, and the position of that impulse in the train. The stimuli were constant currents of long duration of the strength shown in the figure. The nerve fibre always stopped discharging before the end of the stimulation.

E. *The action current of fibre bundles*

In some cases the action currents were led off from fibre bundles containing the fast and the slow fibre for the adductor of the claw and a number of thin sensory fibres. Different results were obtained; sometimes the fibres of the bundle discharged asynchronously on long-lasting currents. In these cases irregular deflexions were observed in which the discharge of the motor fibre could be recognized. In other preparations synchronization of presumably all the nerve fibres took place (see Fig. 11). Two kinds of repetitive discharges could be distinguished in these curves. One fast, short deflexion, which must be due to the discharge of the slow fibre and a less sharp deflexion following the quick one, which must be caused by the discharges of a large number of thin sensory fibres in the bundle. As all our experience points to the difficulty of setting up a series of impulses in the twitch fibre, it can be safely assumed that the repetitive discharge is not located in the twitch fibre. In some cases there was evidence that at the beginning of the discharge the twitch

fibre contributed to the first fast spike, as this was higher than the following ones. In the course of a discharge irregularities are sometimes observed. In such a case the impulse in the slow fibre is not always followed by a single volley of the sensory fibres, or several volleys in the sensory fibres are not preceded by discharges in the slow fibre.

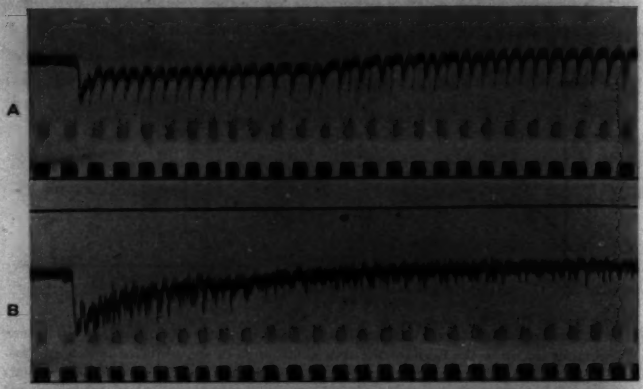

Fig. 11. *Cambarus*. Action currents of a small nerve bundle containing the fibre for the slow contraction on the make of a long direct-current stimulation. A. The discharges of the majority of the fibres are synchronized. One can distinguish the quick action currents of the fibre for the slow contraction from the action currents of the slow-conducting sensory fibres. Notice that sometimes the discharge of most of the sensory fibres is absent. B. Asynchronous discharge of a fibre bundle. The discharge of the fibre for the slow contraction can be recognized in some places.

In small nerve bundles, containing the twitch fibre, the chronaxie of the first quick deflexion has been determined. This value was found to be 0·5σ (average of three preparations), certainly not shorter than the chronaxie of the first step of the twitch. This is another proof that the twitch is caused by only one impulse.

IV. Discussion

That in normal preparations the first step of the twitch is caused by one nerve impulse of only one fibre explains why this contraction shows such a striking all-or-none relation. In preparations of regenerated claws intermediate contractions may be obtained, but it has been shown

that in such preparations more than two fibres for the adductor could be demonstrated, and it is likely that the intermediate contraction is due to two twitch fibres, which have a slightly different threshold. As other crustaceans show the same striking all-or-none relation as does *Cambarus*, and normally do not show intermediate contractions, the explanation might be the same, viz. that these muscles too are innervated by only one twitch fibre. A further investigation seems, however, necessary. The innervation of the adductor in *Astacus fluviatilis* and *Homarus vulgaris* as described by Hoffman [1914] may very well be in agreement with this point of view. He found at most five axons of which (in *Astacus fluviatilis*) two were thick ones. These may be comparable with our fibres for the twitch and the slow contraction. If we assume that in *Astacus fluviatilis* the first step of the twitch is caused by one nerve impulse only, we have to allow that the chronaxie of that fibre is much longer ($1{\cdot}2\sigma$) than in *Cambarus* ($0{\cdot}3\sigma$). There are also other arguments against considering the chronaxie of the twitch in *Astacus fluviatilis* as a pseudo-chronaxie. In *Cambarus* we found that stimulation with strong direct current gives rise to a new impulse in the twitch fibre every 2σ after the preceding one. In *Astacus fluviatilis* this interval seems to be longer (about $4{\cdot}5\sigma$), but also roughly constant. This shows that during the short time needed to set up the first step there would not be time for such an interval, and thus it is very unlikely that two nerve impulses would be set up by this time.

It is clear that the twitch in these animals is very much like the contraction of a single motor unit of vertebrate striated muscle. The mechanism of the slow contraction with the growth of its action currents is unexplained and may not be comparable with any other known nerve-muscle preparation. The fact that the slow contraction and the twitch are evoked by two different axons brings up the question whether there are also two kinds of muscle fibres for these contractions or whether both contractions take place in the same fibre. All that can be said at present is that histological preparations show that the two nerve fibres apparently end in the same muscle fibres. It is certain that the muscle does not consist of a slow and a fast part as in the adductor muscle of some bivalved molluscs.

We can now discuss the way in which other investigators have tried to explain the mechanism of the slow contraction. According to Tonner [1933*a*, *b*] the slow contraction is caused by a peripheral tone mechanism. The reflex arc would start in the sense organs in the membranes covering the joints, from which an axon would split T-shaped towards the

muscle and towards the centre, and an axon reflex in this system would cause the peripheral tone. The total absence of any branching of the slow fibre along its course in the mero- and carpopodite, and the fact that this fibre is the only one that ever gives a slow contraction, make Tonner's explanation improbable. We are of the opinion that all contractions are normally of a central origin. The contractions occurring on mechanical stimulation after the claw has been cut will have to be explained either, as Barnes [1932] has done, as the result of stretching the nerve fibres, or by assuming that sensory fibres are stimulated, the resulting action currents changing the demarcation potential around the cut end of the nerve. In the latter case this change in the demarcation potential would act as an electrical stimulation for the motor fibres. It will also be clear that our experiments are in disagreement with the conception of Du Buy [1935] that the double innervation of this crustacean muscle is comparable with the double innervation of certain vertebrate smooth muscles. There is, for instance, no evidence for a change in resting length of the adductor; after each fast or slow contraction the muscle relaxes completely. As in striated vertebrate muscle the relaxation time is dependent on fatigue.

The action currents of the slow nerve fibre prove that this fibre belongs to the fast-conducting fibres in the nerve, as is in accord with its size. The slow-conducting fibres to which Monnier & Dubuisson [1931] had assigned the function of evoking the slow contraction are presumably not motor fibres, a possibility which has been already pointed out by Wiersma [1933] and by Jasper [1933].

The action currents of the motor nerve fibres show, that but for the greater tendency to give repetitive discharges, these fibres do not seem to possess properties different from those of vertebrate nerves. In the twitch fibres repetitive discharges are difficult to obtain, and they approach in this respect the motor nerves of a frog. The other properties distinguishing the crustacean nerve muscle preparations must be localized in the structures that are involved in the transmission of the nerve impulse to the muscle and in the muscle itself.

V. Summary

A method is described for making single nerve-fibre preparations of crustacean motor axons.

It is shown that the adductors of *Cambarus clarkii* and of *Astacus trowbridgii* are innervated by two motor axons. One of these axons reacts with a single nerve impulse to a single induction shock and causes

a twitch contraction. This contraction is all or none. This axon has in *Cambarus* a mean diameter of 58μ. The chronaxie of this fibre as measured by the muscle contraction or the nerve action current is short, 0·3–0·5σ. If it has not been exposed by dissections this fibre may react with more than one impulse to stimuli above a certain strength. This causes the muscle to give summated contractions which are called the higher steps of the twitch. The strength-duration curves and the pseudo-chronaxies of two of those steps have been measured. Summation of two single impulses following each other at different time intervals was studied; the corrected least interval was between 2 and 3σ; the longest time for which summation is obtainable was more than 40σ and was shorter when a single impulse no longer had any effect.

The nerve fibre evoking the slow contraction has a mean diameter of 36μ. This fibre differs from the twitch fibre in that long direct-current stimulations set up a series of impulses with a maximum frequency of 200 per sec. The nerve action currents of this fibre are all of the same height. The slow contraction results only when a number of impulses have reached the muscle. The muscle-action currents accompanying the slow-contraction grow in size, but always stay much smaller than the action current of the first twitch.

In regenerated claws more motor axons may be present and this causes several divergent results.

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612.63.031.1:612.621

THE ACTION OF PROGESTERONE ON THE UTERUS OF THE RABBIT AND ITS ANTAGONISM BY ŒSTRONE

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(*Received May* 7, 1936)

When an extract of the corpus luteum is injected into rabbits, the uteri of these animals subsequently suspended in physiological solution fail to react to the oxytocic hormone of the posterior pituitary lobe [Knaus, 1929]; a similar inhibition is also observed when the contractions of the uterus are registered *in situ* [Robson, 1935 *b*]. For the sake of clarity this phenomenon will be referred to as desensitization of the uterus. Investigations with various fractions of a crude extract of the corpus luteum have shown that the degree of desensitization to oxytocin *in vitro* does not always run parallel with the effect (progestational proliferation) of the luteal hormone on the endometrium [Robson & Illingworth, 1931; De Fremery, Luchs & Tausk, 1932], and have suggested the possibility that the active factors responsible for the actions on the uterine muscle and endometrium respectively may not be identical. In view of the recent isolation and partial synthesis of progesterone, the active principle of the corpus luteum, it was decided to determine to what extent the known actions of the luteal extracts on the uterus could be attributed to this substance and the first part of this paper records the results obtained.

Progestin is effective in producing its actions on the uterus (progestational proliferation of the endometrium and desensitization of the muscle) only when the uterus has been subject to the action of œstrin immediately previously [Allen, 1930; Hisaw & Leonard, 1930; Robson, 1932]. On the other hand, the action of progestin on the uterine endometrium can be prevented by the simultaneous injection of

œstrin; but the effect on the uterine muscle is not easily overcome by œstrin [Robson & Illingworth, 1931; Leonard, Hisaw & Fevold, 1932]. These data were obtained with comparatively crude preparations of the ovarian hormones, and in the present experiments the question of the antagonism of these hormones has been quantitatively investigated, using crystalline œstrone and progesterone.

Methods

The experiments were performed on thirty-one mature female rabbits of various breeds. The weights are given in Table I. All animals were ovariectomized and the investigation on the uterus done 12 or 13 days after operation. The animals may be divided into three groups:

(1) Fourteen rabbits injected with œstrone and then with progesterone; 0·01 mg. of œstrone in 0·1 c.c. oil was given daily for 7 or 8 days following ovariectomy, and progesterone, also dissolved in oil, was administered during the subsequent 4 days in eight doses on the morning and evening of each day.

(2) Fifteen rabbits were injected with œstrone for 7 days as in group (1), and for 4 days with both œstrone and progesterone; doses of both ovarian hormones were given twice daily during this latter period (except in the case of Ra 656), the total doses administered being shown in Table I. Ra 656 received eight injections of progesterone, but the œstrone was given in two doses on the first day and at 2-hourly intervals during the last 3 days of the 4-day period (no injections were given during the nights); the total amount of œstrone administered during these 4 days was 0·17 mg. in seventeen injections of 0·01 mg.

(3) Two control animals (Ra 657, 661) received only the preliminary œstrone injections over 7 days, and no injections during the subsequent 4 days which preceded the experiment.

The details of the experiment on the uterus *in vitro* and *in vivo* are exactly similar to those previously reported [Robson, 1935 *b*].

The degree of progestational proliferation was determined upon sections of at least two parts of the uterus; no appreciable discrepancy between the proliferation shown by the sections obtained from any one animal was noted. A proliferation of 3·0 is equivalent to the maximum observed at the height of pseudo-pregnancy; 1·0 is the least clearly definable proliferation and 2·0 is a degree of proliferation intermediate between 1·0 and 3·0.

The crystalline progesterone (prepared from stigmasterol) was very kindly supplied by Dr M. Tausk of N. V. Organon. An extract of the

posterior-lobe "pituitrin", and the separate oxytocic and pressor fractions "pitocin" and "pitressin" (for which I am indebted to Dr White, of Parke, Davis and Co.) were used in this investigation.

Results

The results of all the experiments are given in Table I. The data under "œstrone injections" refer only to œstrone administered during the last 4 days preceding the experiment, all the animals having previously received seven or eight daily doses of the hormone following bilateral removal of the ovaries. The reactivity to the various drugs is expressed as the minimum dose capable of eliciting a motor effect. When the experiment was performed *in vitro* the data refer to the dose added to 100 c.c. of Ringer-Locke, while the results given for the *in vivo* investigations refer to the amount of drug injected intravenously. Since the blood volume of the animals used was not much over 100 c.c. the concentration of oxytocin produced by the addition of a given quantity of the posterior-lobe hormone to the bath or by its intravenous injection would be about the same. In certain cases small doses of a drug had no effect, but when the dose was increased inhibition, and not contraction, was produced. These figures are given in italics in Table I and refer, therefore, to a dose of the given drug capable of producing an inhibitory action.

(1) *Animals injected with progesterone only, after the preliminary treatment with œstrone.* These animals were injected with total doses of progesterone varying from 5·0 to 0·25 mg. over the period of 4 days. It will be noted that the uterine endometrium of all the rabbits treated with 0·75 mg. or more showed a progestational proliferation of 2·0 or more; in seven out of nine of these animals the proliferation was greater than 2·0 and of the same order as that seen at the height of pseudo-pregnancy, or greater. In the three animals injected with 0·5 mg. the proliferation was quite appreciable, and even 0·25 mg. was sufficient to produce a minimum proliferation in the endometrium of one of the animals (Ra 634) to which that dose was administered.

The measurements on the uterine muscle showed that progesterone had markedly decreased the reactivity to oxytocin. Thus in all but one (Ra 623) of the animals injected with 0·75 mg. or more of the luteal hormone 1·0 unit of oxytocin in 100 c.c. of Ringer-Locke failed to produce any effect, and even in Ra 623 the motor reaction to 1·0 unit was slight; 0·5 mg. of progesterone also produced some degree of desensitization. The desensitizing effect of progesterone was not so con-

TABLE I. Showing the reactivity to the posterior lobe extract and its oxytocic and pressor fractions of the uterine muscle of all the experimental animals. The reactivities are expressed as the minimum dose added to 100 c.c. of Ringer-Locke or injected intravenously capable of eliciting a motor effect. Figures in italics refer to doses producing inhibition of the uterine muscle. The data given in columns 3 and 4 refer only to œstrone injected during the last 4 days before the experiment; all animals received, in addition, seven or eight injections of 0·01 mg. of œstrone during the previous 7 or 8 days. For details of œstrone injections of Ra 656 see text. The injection of 1·0 unit of oxytocin intravenously in this animal produced a contraction followed by a relaxation. In the case of Ra 590 the addition to the bath of 1·0 unit of oxytocin produced a contraction followed by a relaxation.

Animal No. Ra	Wt. kg.	Œstrone injections mg.	Progesterone injections mg.	Progestational proliferation	Reactivity to pituitrin *In vitro* Longitudinal	Reactivity to pituitrin *In vitro* Circular	Reactivity to pituitrin *In vivo*	Reactivity to oxytocin *In vitro* Longitudinal	Reactivity to oxytocin *In vitro* Circular	Reactivity to oxytocin *In vivo*	Reactivity to vaso-pressin *In vitro* Longitudinal	Reactivity to vaso-pressin *In vitro* Circular	Reactivity to vaso-pressin *In vivo*	Spontaneous activity *In vitro* Longitudinal	Spontaneous activity *In vitro* Circular	Spontaneous activity *In vivo*
657	2·0	—	—	—	—	—	—	0·1	—	0·10	0·5	—	*1·0*	0·2, 0·5	—	0·3
661	2·1	—	—	—	—	—	—	0·1	—	0·05	0·2	—	0·2	0·3, 1·0	—	0·7
589	2·05	—	5·0	2·0	—	—	—	>1·0	—	>1·0	—	—	—	0·5, 0·5	—	1·0
590	2·05	—	5·0	3·5	—	—	—	>1·0 *>1·0*	—	>1·0	—	—	—	0·9, 1·5	—	0·8
600	2·0	—	2·0	3·0	—	—	—	>1·0	—	0·2	—	—	—	0·8, 1·2	—	0·7
601	2·0	—	2·0	3·5	—	—	—	>1·0	—	1·0	—	—	—	5·0, 8·0	—	1·0
604	2·5	—	1·0	4·0	—	—	—	>1·0	—	0·2	—	—	—	5·0, 7·5	—	0·8
633	2·8	—	1·0	2·0	—	—	2·0	1·0 (slight)	—	>1·0	—	—	—	0·5, 2·5	—	0·7
647	1·65	—	0·75	2·5	*5·0*	—	2·0	>5·0	—	>1·0	—	—	—	11·0, 15·0	—	0·4
624	2·3	—	0·75	3·0	>5·0	—	*2·0*	>5·0	—	>0·5	—	—	—	0·8, 1·0	—	0·7
633	2·05	—	0·75	3·0	*1·0*	—	—	>1·0	—	1·0	—	—	—	4·0, 10·0	—	0·7
629	1·85	—	0·5	2·0	>1·0	—	1·0	>1·0	—	0·5	—	—	—	0·3, 1·0	—	0·6
628	1·75	—	0·5	2·0	—	—	—	>0·5	—	>1·0	—	—	—	0·3, 1·0	—	0·5
635	1·75	—	0·5	1·5	—	—	—	0·5	—	0·2	—	—	—	2·0, 5·0	—	0·3
634	2·15	—	0·25	1·0	—	—	—	0·5	—	0·5	—	—	—	2·0, 10·0	—	1·4
636	1·95	—	0·25	Nil	—	—	—	0·2	—	0·05	—	—	—	1·3, 3·0	—	0·2
637	1·7	0·2	0·75	Nil	1·0	—	>1·0	>1·0	—	>1·0	—	—	—	9·0, 10·0	—	3·0
640	1·7	0·12	0·75	Nil	—	—	—	0·1	—	>0·5	—	—	—	5·0, 11·0	—	1·9
639	1·7	0·08	0·75	Nil	—	—	—	0·2	—	>0·3	—	—	—	9·0, 11·0	—	3·0
642	1·8	0·04	0·75	Nil	1·0	—	5·0	0·5	—	>1·0	—	—	—	10·0, 24·0	—	1·8
644	1·85	0·02	0·75	Nil	5·0	—	*1·0*	>1·0	—	>1·0	—	—	—	11·0, 12·0	—	0·7
648	1·8	0·01	0·75	Nil	—	—	*1·0*	0·5	—	*1·0*	—	—	—	9·5, 24·0	—	0·5
618	2·15	0·005	0·75	1·0	1·0	1·0	*1·0*	>1·0	>1·0	>1·0	0·2	1·0	*1·0*	1·0, 7·0	2·0	0·3
653	2·15	0·0025	0·75	1·0	1·0	1·0	*1·0*	>1·0	>1·0	>1·0	2·0	>2·0	*<2·0*	6·0, 12·0	3·0	0·6
643	1·8	0·08	1·5	Nil	1·0	—	*1·0*	0·5	—	>0·5	—	—	—	10·0, 11·0	—	1·5
645	1·75	0·04	1·5	Nil	1·0	—	*1·0*	>1·0	—	>0·5	—	—	—	7·0, 9·0	—	1·0
649	2·2	0·01	1·5	1·0	5·0	5·0	*1·0*	>1·0	—	—	4·0	—	*<2·0*	1·5, —	0·6	0·1
650	2·0	0·005	1·5	3·0	1·0	>1·0	—	1·0	—	>1·0	>2·0	>2·0	*1·0*	0·7, 1·1	1·1	0·2
654	1·65	0·0025	1·5	3·0	1·0	>1·0	*1·0*	>1·0	—	>1·0	0·4	>2·0	*<1·0*	4·0, 4·5	3·5	0·3
655	1·9	0·01	3·0	3·0	1·0	>1·0	>3·0	>1·0	—	—	>2·0	—	—	0·0, 1·3	1·2	0·0
656	2·05	0·17	0·75	Nil	—	—	—	0·1	—	1·0	0·8	—	*<0·5*	1·0, 6·0	—	0·6

sistent when the measurements were made on the uterus *in situ* and was only slight in two (Ra 604, 600) out of the nine animals injected with 0·75 mg. or more. In several animals the reactivity to the unfractionated extract of the posterior pituitary lobe "pituitrin" was determined. This either produced no effect or inhibited the muscle *in vitro*; on the intact uterus a motor reaction was produced in three out of four experiments, while in the fourth the intravenous injection of 2 units of pituitrin brought about inhibition.

(2) *Animals injected simultaneously with progesterone and œstrone, after the preliminary treatment with œstrone.* These experiments were planned to determine quantitatively the relationship existing between the actions of œstrone and progesterone and may be divided into four groups, namely: (*a*) Eight animals which received 0·75 mg. of progesterone during the 4 days preceding the investigations on the uterus, and also doses of œstrone, varying in the different animals from 0·2 to 0·0025 mg. in the same period. (*b*) Five rabbits which received 1·5 mg. of progesterone and doses of œstrone varying from 0·8 to 0·0025 mg. on the 4 days preceding the experiment. (*c*) One rabbit which received 3·0 mg. of progesterone and 0·01 mg. of œstrone. (*d*) One animal which received 0·75 mg. of progesterone and repeated doses of œstrone totalling 0·17 mg.

It was found that the effect of 0·75 mg. of the luteal hormone on the uterine endometrium could be completely inhibited by a total dose of 0·01 mg. of œstrone simultaneously administered; even 0·0025 mg. of œstrone produced an appreciable inhibition of the progestational action of 0·75 mg. of progesterone so that the resulting proliferation was 1·0 instead of 2·0 or more (see Table I). When the dose of the luteal hormone was increased to 1·5 mg. a complete inhibition of its action on the endometrium was obtained only when 0·04 mg. of œstrone was simultaneously injected, while the administration of 1·5 mg. of progesterone and 0·01 mg. of œstrone (which completely inhibited the progestational effect of 0·75 mg. of progesterone) resulted in a proliferation of 1·0. No inhibition of the action of 1·5 mg. of progesterone on the endometrium was detectable with doses of œstrone of 0·0025 and 0·005 mg. When the dose of progesterone was again doubled (3·0 mg.) no detectable inhibition of the action on the endometrium was obtained when 0·01 mg. of œstrone was simultaneously injected.

The results on the uterine reactivity *in vitro* show that œstrone may, to some extent, decrease the desensitizing effect of progesterone. This can best be seen by reference to Table I, column 11, comparing the groups of animals Ra 589–636 and Ra 637–656. The antagonistic effect

of œstrone on the action of progesterone on the muscle is, however, seen only in the minority of animals; moreover, it is not marked, as even the repeated administration of œstrone at 2-hourly intervals in animal Ra 656 failed to produce a reactivity to oxytocin higher than 0·1 unit per 100 c.c. of solution. The above effects were obtained when the measurements were made on the uterus suspended in physiological solution; but no interference with the desensitizing action of progesterone by œstrone was observed when the measurements were performed on the uterus *in situ*. For none of the animals receiving eight doses of œstrone simultaneously with progesterone showed any appreciable effect on the uterus when the oxytocin was injected intravenously. In rabbit Ra 656, which received repeated injections of œstrone, the intravenous injection of 1·0 unit of oxytocin produced some motor effect, but this was followed by an inhibition.

The results obtained with pituitrin *in vitro* and *in vivo* in the animals receiving both œstrone and progesterone agree with those obtained with oxytocin; for the desensitizing action of the luteal hormone was, to some extent, prevented by œstrone, when the experiments were performed *in vitro* while no such interference was observed when the investigations were performed on the uterus *in situ*. Indeed the intravenous injection of pituitrin into rabbits treated simultaneously with progesterone and œstrone frequently produced an inhibition of the uterus, as shown by a decrease in the rhythmic contractions and a fall in the tone. Such an inhibition of the uterus had been observed only once with oxytocin (Ra 648; the uterus of Ra 656 also showed some inhibition following a preliminary motor effect), and it appeared possible that the inhibitory action of the posterior lobe extract was due to its content of pressor hormone; this was found to be the case as the intravenous injection of vasopressin produced inhibition of the uterus in all six animals (Ra 618, 653, 649, 650, 654, 656) in which this effect was investigated. This inhibitory action of vasopressin on the uterus of animals treated with progesterone and œstrone, which was sometimes very marked, is illustrated in Fig. 1, which also shows the absence of any reaction to oxytocin (Ra 618).

The fact that the posterior-lobe extract and its pressor fraction brought about marked inhibitory reaction of the uterus in the intact animal, while producing contractions of the uterus suspended *in vitro* led to a further investigation of this difference. It appeared possible that the motor reactions of the uterine muscle to vaso-pressin *in vitro* might be exerted only on the longitudinal muscle, while the circular muscle might be inhibited by the posterior lobe extracts. In order to investigate this

a portion of the uterus was removed and its reaction determined by use of the Trendelenburg method. It was found in some experiments

Fig. 1. Illustrating the inhibition of the uterine muscle *in vivo* by vaso-pressin (Rs 618). First signal: 1·0 unit pitocin injected intravenously. Second signal: 1·0 unit pituitrin injected intravenously. Third signal: 2·0 units of pitressin injected intravenously. Fourth signal: 1·0 unit of pitocin injected intravenously. Intra-uterine pressure = 10·5 cm. of water. Time intervals = 1 min.

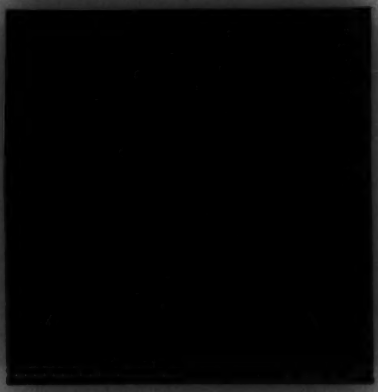

Fig. 2. Illustrating the contraction of the longitudinal muscle to pituitrin *in vivo* and the absence of any contraction of the circular muscle (Rs 654). Trendelenburg registration. Upper tracing = circular muscle. Lower tracing = longitudinal muscle. Signal: 1·0 unit of pituitrin added to the bath (100 c.c.). Intra-uterine pressure = 2·0 cm. Time intervals = 1 min.

that the addition to the bath of pituitrin or pitressin caused a contraction of the longitudinal muscle without producing any change in the record of the circular muscle. An example of such a discrepancy is illustrated

in Fig. 2 from animal 654. At the signal 1·0 unit of pituitrin was added to the bath and this was followed by a marked contraction of the longitudinal muscle but no definite effect on the circular muscle.

Spontaneous activity

The data for the spontaneous rhythmic activity exhibited by the uterus *in vitro* and *in vivo* are given in Table I, columns 18, 19 and 20. As the experimental conditions have been kept as constant as possible the results for the various experiments are comparable, though the actual figures represent measurements on an arbitrary scale (direct measurements in cm. on the smoked records).

It will be seen that the height of the spontaneous activity *in vitro* shows considerable differences in the various experiments; at the same time the results show that, on the whole, the rhythmic contractions are larger for the uteri of animals treated with œstrone and progesterone than for those of animals treated with progesterone only.

The rhythmic activity *in vivo* of the uterus of animals injected with œstrone for 7 days following ovariectomy and then left untreated for the ensuing 4 days (Ra 657, 661) is small, as is the activity in animals treated with progesterone following the preliminary œstrone injections. An examination of the degree of spontaneous activity *in vivo* in animals treated with progesterone and œstrone simultaneously (following the preliminary injections over 7 days with œstrone) shows that, with a given dose of progesterone, the rhythmic contractions of the uterus tend to increase as the amount of œstrone given at the same time is increased. There is also support for the view that the height of the contractions decreases as the dose of progesterone is increased (the amount of œstrone remaining constant), as can be seen from the small number of relevant results collected in Table II.

TABLE II. Showing the relation between the total dose of progesterone and the spontaneous activity exhibited *in vivo*.

Animal No.	Total dose of progesterone mg.	Total dose of œstrone mg.	Spontaneous activity *in vivo*
639	0·75	0·08	3·0
643	1·5	0·08	1·5
642	0·75	0·04	1·8
645	1·5	0·04	1·0
648	0·75	0·01	0·5
649	1·5	0·01	0·1
655	3·0	0·01	Nil

Discussion

The results of these experiments give information on the action of crystalline progesterone on (1) the uterine endometrium, and (2) the uterine muscle of the rabbit.

The data on the endometrium show that a total dose of 0·75 mg. of the luteal hormone produces a degree of progestational proliferation equivalent to that seen at the height of pseudo-pregnancy (3·0). This is an amount similar to that necessary to produce an equal degree of proliferation in the immature rabbit when the test is performed by the method of Clauberg. When the hormone is injected into the mature animal ovariectomized after mating (method of Corner & Allen), then 1·5 mg. are necessary to produce a proliferation of 3·0 [Woods & Young, 1936]. Results recently published by Fels [1935] and by Woods & Young [1936] working in Corner's laboratory show that the test on the immature animal has the disadvantage that a wide degree of variation of the result produced on the endometrium is observed in different animals. Hence a comparatively large number of rabbits have to be used for a satisfactory standardization. It is possible that differences in the pituitary activity of the immature animals may account for the variations in the response of the endometrium to progesterone, as there is evidence that the endometrium of the hypophysectomized rabbit is less responsive to threshold doses of the luteal hormone [Robson, 1935 *a*].

The test on the mature rabbit, performed after mating and ovariectomy, or after ovariectomy and a series of œstrin injections, does not suffer from this disadvantage as shown by the results of Woods & Young [1936] and by the present experiments. Moreover, there appears to be no variation in the mature rabbit in the different parts of the same uterus, as has been described for the immature animal by McPhail [1934]. The method of assay on the mature ovariectomized rabbit treated with œstrin has the further advantage that it renders the endometrium as sensitive as in the immature animal, since in both 0·75 mg. produces a full degree of progestational proliferation; moreover, mating is not necessary and the test need not be performed on œstrous rabbits.

The studies on the antagonism between œstrone and progesterone on the endometrium show that the action of the luteal hormone can be completely inhibited by 1/75th of its weight of œstrone; and some degree of inhibition is still apparent when the weight of progesterone

given is more than 200 times that of œstrone simultaneously administered. When, however, the ratio between the two hormones is raised to 1:300 no inhibitory effect may be demonstrated (Ra 650 and 655). In as far as can be judged from the results obtained the ratio of the two hormones given is the essential factor in determining what degree of luteal proliferation is produced when œstrone plus progesterone (in adequate amounts) act on the uterine endometrium.

The question whether progesterone can produce desensitization of the reaction of the uterine muscle to oxytocin is answered in the affirmative. This applies both to the uterus investigated *in vitro* and *in vivo*. The results obtained *in vitro* are in agreement with those of Makepeace, Corner & Allen [1936]. In view of the results of Robson & Illingworth [1931] and of De Fremery, Luchs & Tausk [1932] it still seems possible that there is present in crude extracts of the corpus luteum a substance capable of producing desensitization and not progestational proliferation.

The administration of œstrone together with progesterone has, in a number of experiments, produced some increased reactivity of the muscle *in vitro*; nevertheless, the uterus did not in any of the experiments respond to small doses of the posterior lobe hormone even though in one case (Ra 656) repeated injections of œstrone had been given. As it has been previously shown [Robson, 1933] that such œstrone injections by themselves lead to a high uterine sensitivity, it can therefore be concluded that the desensitizing action of progesterone on the uterine reactivity measured *in vitro* is only occasionally, and then only partially, antagonized by œstrone injected at the same time. On the other hand, œstrone appears to enhance the desensitizing action of progesterone on the uterine muscle *in situ*, since the uteri of all the animals which received eight doses of the two hormones on the 4 days preceding the experiment failed to show any motor reaction when oxytocin was injected intravenously (Ra 637–655, Table I).

It is difficult to determine at present the exact significance of the experiments on the uterine reactivity *in vitro* and *in vivo* produced by the simultaneous injections of œstrone and progesterone. Nevertheless, one fact emerges definitely, that the optimum desensitization of the uterus *in situ* results from the combined action of the two ovarian hormones. Since the condition of the uterus *in vivo* is the important one from the functional aspect of maintenance of pregnancy, it follows that in this respect, as in a number of others, the optimum effect of progesterone is achieved in synergism with œstrone.

The conclusions arrived at from a previous series of experiments [Robson, 1935 *b*], that the reactivity to oxytocin of the uterine muscle *in vitro* offers an accurate indication of the state of the uterus *in situ*, now requires certain qualifications, for in the absence of any œstrogenic hormone the desensitizing effect of progesterone will completely develop only for the muscle studied *in vitro*; on the other hand, medium doses of œstrone will partially antagonize the desensitizing action of progesterone on the isolated uterine muscle, while not interfering with the development of desensitization of the uterus *in situ*. It appears probable that the almost constant agreement found in the previous series of experiments was due to the use of fairly crude luteal extracts, which would probably contain sufficient œstrone for the development of complete desensitization *in vivo* but not sufficient œstrone to overcome the desensitizing effect of progesterone *in vitro*. At the same time it must be pointed out that the discrepancies obtained even in the present experiments between the reactivities *in vitro* and *in vivo* were never very marked.

The effects obtained with vaso-pressin are of interest as they suggest that the treatment of the uterus with œstrone sensitizes it to the inhibitory action of the pressor hormone. Similar results are being obtained in experiments now in progress on the uterus of the cat [Robson & Schild] and it is hoped to discuss the question more fully in the near future.

Summary

Mature rabbits were ovariectomized and injected with œstrone for 7 or 8 days; during the ensuing 4 days they received either (1) no injection, or (2) injections of progesterone, or (3) injections of both progesterone and œstrone. On the day after the last injection the following observations were made: (1) histological condition of the uterine endometrium; (2) reactivity of the uterine muscle *in vitro* and *in vivo* to an extract of the posterior pituitary lobe and its oxytocic and pressor fractions; (3) spontaneous rhythmic activity of the uterine muscle *in vitro* and *in vivo*.

A total dose of 0·75 mg. of progesterone, or more, usually produced a degree of progestational proliferation equivalent to that observed at the height of pseudo-pregnancy. The animal variation was small. The advantages of this method for the assay of progestin are discussed.

The effect of progesterone on the endometrium can be completely inhibited by the simultaneous injection of 1/75th of its weight of œstrone: some inhibition may be obtained with 1/300th of its weight of œstrone.

Allowance must be made for this in the assay of luteal extracts containing small amounts of œstrin.

Progesterone produces desensitization of the reaction of the uterine muscle to oxytocin. When œstrone is simultaneously administered the desensitization *in vitro* may be somewhat decreased, but full desensitization of the muscle *in situ* is consistently obtained.

Vaso-pressin may produce inhibition of the uterine muscle *in situ*; this inhibition was always obtained in animals treated with œstrone.

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MECHANISM OF THE PERIPHERAL VASCULAR RESPONSES TO CHANGES IN BLOOD GAS TENSION IN MAN[1]

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(*Received May* 13, 1936)

Changes in the cardiovascular system coincident with alterations in blood gas tension have been demonstrated repeatedly in man. In animals it has been possible to analyse the various components of these changes. In man, however, the role played by the nervous system in these changes has not been fully elucidated. It is the purpose of this paper to record the responses of the peripheral circulation of man to changes in blood gas tension and to analyse the nervous mechanisms involved in these responses.

Method

Twenty-nine subjects were examined, of these twenty-one were hospital patients and eight were normal young post-graduates. All the normal subjects of this investigation were shown to have normal peripheral vaso-motor responses to changes in blood temperature, to sensory stimuli, and to alteration of respiratory rhythm, before any attempt was made to investigate their reactions to changing blood gas tension. Fourteen of the patients examined were suffering from the effects of single and discrete neurological lesions. The remaining seven patients were, for the purposes of this investigation, normal. The extent of the neurological lesions was supported in each case by a clear-cut clinical picture. In several cases the diagnosis had been confirmed at operation. They all had normal vaso-motor responses in the limbs which were unaffected by the nervous lesions. No patients showing evidence of hypertension or of systemic arterial disease were included.

[1] Work undertaken on behalf of the Medical Research Council while Dr Williams held a Halley Stewart Research Fellowship.

The subjects sat or lay at rest. The initial condition of their peripheral vascular bed was altered by raising or lowering the blood temperature by immersion of the legs in tanks of hot (44° C.) or cold (about 14° C.) water. In this way general peripheral vaso-constriction or vaso-dilatation could be produced and sustained for long periods of time.

A finger or toe plethysmograph was used to record changes in digital volume. Care was taken to prevent any constriction of the digit by the proximal end of the plethysmograph, and to avoid any movement of the digit within the plethysmograph. The alteration in digit volume was recorded photographically, the method permitting simultaneous records to be obtained from more than one digit [Bolton *et al.* 1936].

Variations in the tension of carbon dioxide and oxygen in the blood were produced by breathing differing and known gaseous mixtures from a Douglas bag system. The procedure was previously explained to the subjects and care was taken not to disturb them in order to exclude the rapid vaso-motor changes which are known to occur as a result of various sensory stimuli. The factors investigated were increase and decrease of carbon dioxide partial pressure, increase and decrease in oxygen partial pressure, and increase of carbon dioxide with simultaneous decrease of oxygen partial pressure. By controlling the blood temperature the changes were investigated in subjects whose peripheral vessels were initially dilated by warmth, or were in a state of general constriction due to cooling. Systolic blood-pressure readings were taken as frequently as possible during the period of the experiments with a mercury sphygmomanometer by auscultation. The time of each reading was marked by a signal on the photographic record.

A. Increase of carbon dioxide partial pressure in the blood was produced by respiring through a Douglas bag system with Douglas valves. The bag contained a mixture of 10 or 20 p.c. carbon dioxide in oxygen, and the mouthpiece was fitted comfortably to the subject. After a sufficient interval had elapsed to accustom the subject to the mouthpiece and to make certain that breathing through the system caused no vaso-motor response, the tap was quietly turned and the subject inhaled the gaseous mixture until signs of dyspnœa were apparent. The tap was then quietly opened to air. After the records had been completed the mouthpiece was removed. The apparatus and signals were so arranged that the subject was unaware of the turning of the tap or of the signals being given.

B. Decrease in carbon dioxide tension was brought about by prolonged moderate overbreathing. This was continued for periods up to 15 min. If hyperventilation were carried out without excessively deep breathing, it

was possible to exclude the vaso-constriction in response to deep inspiration, which has been described [Bolton *et al.* 1936].

C. Increase in oxygen tension was produced by inhaling oxygen from a Douglas bag system in a manner similar to that used to produce increased carbon dioxide tension.

D. Decrease in oxygen tension was brought about in two ways, (i) by rebreathing expired air in a closed system in which the inspired air was passed over KOH, and (ii) by breathing pure nitrogen from a Douglas bag.

E. Reduction in oxygen with increase in carbon dioxide tension was accomplished by breath holding or by rebreathing until dyspnœa supervened.

Results

A. Increase in carbon dioxide tension. The subjects breathed known carbon dioxide mixtures for intervals varying from a few seconds, when a slight increase in the depth of respiration occurred, up to several minutes, when a state of transient mental confusion was brought about.

In most cases the tap was turned to air before extreme hyperpnœa supervened. Vaso-motor responses may be elicited with such facility by the sensations of discomfort and distress or by apprehension or fear, that great care was observed in order to eliminate these responses from the results obtained.

An increase in the depth and rate of respiration usually occurred after two to three inhalations of a 10 p.c. carbon dioxide 90 p.c. oxygen mixture had been made, and continued until pronounced hyperpnœa was produced. On opening the system to air the increase in the depth and rate of respiration persisted for periods up to 30 sec. In some cases the depth of respiration increased after the tap had been turned to air. Following this hyperpnœa a rapid decrease in respiratory rate and volume took place, but ultimate return to the normal level occurred more slowly (Fig. 1). Alterations occurred in the digital volume concurrently with these respiratory changes. With the vessels in an initially constricted state no change could be seen in the digital volume during the breathing of the carbon dioxide mixtures. After a varying period of time, and usually following the opening of the system to air, a rapid and progressive increase in digital volume occurred with a corresponding increase in pulse wave (Fig. 1). With the vessels in a state of dilatation the following changes occurred. There was a progressive diminution in the digital volume during the inhalation of the carbon dioxide mixture. This occurred after several inspirations of the mixture had been made, was maintained for some

30–40 sec., and was associated with a decrease in the size of the pulse wave and a slight increase in the pulse rate. These changes were not as profound as those produced by lowering the blood temperature. The decrease in volume was followed by a rapid increase until the initial level was reached (Fig. 2). If the vessels were not initially in a fully dilated state this increase was continued beyond its original level to be followed by a slow return to the original volume after 3–5 min. It could be shown that this

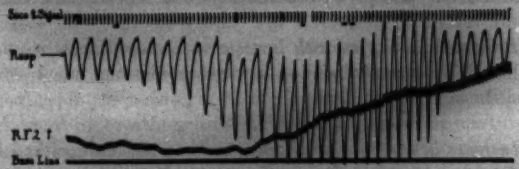

Fig. 1. *Normal.* Inhalation of a mixture of 20 p.c. carbon dioxide in oxygen. Vessels initially constricted. In this and in the following figures respiration, digital volume and the time in seconds are recorded. The letters RF_2 refer to the right second finger. In all cases the arrow points in the direction of increase of finger volume. The first signal indicates the time of commencement of inhalation of the carbon dioxide mixture and the second signal marks the time of its cessation.

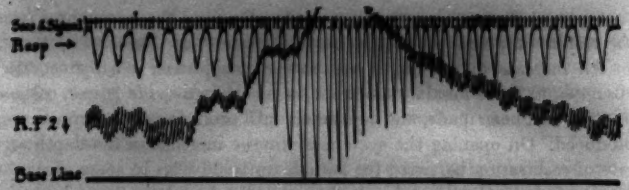

Fig. 2. *Normal.* Inhalation of a mixture of 20 p.c. carbon dioxide in oxygen. Vessels initially dilated. Signals—carbon dioxide mixture on and off.

increase in digital volume was maximal—that is, as great as that produced by prolonged raising of the blood temperature. Although the fall in digital volume which followed inhalation of carbon dioxide mixtures was absent with the vessels in a primary state of constriction and was present to a varying extent with them in a dilated state, the subsequent increase in digital volume was constant in its occurrence and characteristics. This subsequent increase of digital volume had a relatively gradual increment and a very prolonged decrement, and was accompanied by an increase in the amplitude of the pulse wave and by some slowing of the cardiac rate.

The systolic blood pressure was charted against the records of the digital volumes. In each case examined there was an increase in blood pressure during respiration of carbon dioxide mixtures. Using mixtures of 10 and 20 p.c. carbon dioxide the rise varied from 10 to 60 mm. of mercury. This increase occurred after a few breaths of the mixture had been taken and reached its maximum within a minute. The blood pressure then steadily fell to its former level. The increase in blood pressure occurred when the subject was in a cool state with the peripheral blood vessels constricted, and later when the subject was in a warm state with the peripheral blood vessels dilated. In the former condition it occurred without any demonstrable fall in finger volume. In the dilated state it followed upon the slight fall in finger volume. When the blood pressure had reached a relatively high level the digital volume began to increase. This increase was followed closely by the fall in blood pressure. The fall

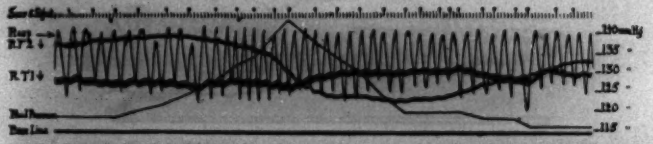

Fig. 3. *Paraplegia.* Inhalation of a mixture of 20 p.c. carbon dioxide in oxygen. Records taken from the right second finger and right great toe. Systolic blood pressure in mm. of mercury. The upper signals refer to times of blood-pressure readings, the lower to the opening and closing of the valves to the gaseous mixture.

in blood pressure was in all cases preceded by the increase in digital volume (Fig. 3). The digit often continued to increase in volume for a short time after the blood pressure had become stable, but it subsequently returned to its initial volume.

The above experiments show, therefore, that when the peripheral blood vessels are dilated an increase in carbon dioxide tension produces an initial decrease in digital volume accompanied by a rise in systolic blood pressure; these changes are followed by a rapid and profound increase in digital volume and decrease in blood pressure. With the vessels in a state of constriction no fall in digital volume takes place, but a rise in blood pressure occurs (Fig. 3). This is again followed by a rapid increase in digital volume, which is often maximal, and by a coincident fall in systemic pressure. Further experiments were carried out to elucidate the mechanisms involved in these peripheral changes.

The following experiment was made in order to try to establish the part played by the arterial and capillary beds in these changes. A subject

immersed his hand in as hot water as could be borne and persisted in re-immersing it for as long periods as could be tolerated. The hand became very flushed, and it was believed that full dilatation of the arteries, arterioles and capillaries of the fingers had been produced. Further, it was thought that the procedure was so drastic that temporary paralysis of the capillaries in full dilatation had been brought about by the liberation of an histamine-like substance in the digits. A plethysmograph was rapidly fitted and the subject breathed 20 p.c. carbon dioxide in oxygen. As has been previously described, a decrease followed by a great increase in digital volume beyond its previous level took place. This was similar to, and synchronous with, the changes in the digit of the other hand, the vessels of which were in a relatively constricted state (Fig. 4). In a further experiment the subject's blood temperature was raised, with consequent general arterial dilatation, and a 10 p.c. carbon dioxide

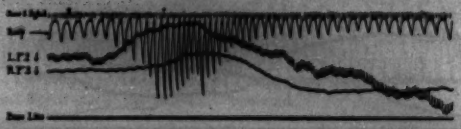

Fig. 4. *Normal.* Inhalation of a mixture of 20 p.c. carbon dioxide in oxygen. Vessels of right finger initially constricted; those of left finger dilated by an histamine-like response to immersion in hot water. Signals—carbon dioxide mixture on and off.

mixture was again breathed. By raising the blood temperature adequately, full arterial and arteriolar dilatation is brought about. The capillary bed, though increased, is not fully dilated as in an histamine reaction. In this case a fall in volume, followed by an increase in volume to its previous level, took place in the finger. The response was similar to that in the previous experiment. The only difference between the two experiments was the degree of dilatation of the capillaries and their ability to contract. Though not conclusive, these two experiments suggest that, as alteration in the capillaries does not affect the response, the arterial, including arteriolar, rather than the capillary bed is mainly concerned with the changes which have been described. Two other factors, namely cardiac output and venous content, may also have played a part. These will be discussed later.

In order to determine the mechanism by which changes in finger volume were brought about in response to the alterations in the carbon dioxide tension of the blood, the responses of patients suffering from the

effects of various destructive lesions of the central nervous system were investigated.

Two subjects with conclusive evidence of a complete transverse lesion of the spinal cord were examined. One had a lesion causing complete destruction of the cord below the tenth dorsal spinal segment. There was no evidence of reflex activity below the level of the lesion. There was evidence that the preganglionic sympathetic neurones were functionally inactive. The other patient suffered from transverse myelitis which had caused complete physiological discontinuity of the cord at the sixth dorsal segment, leaving a reflexly acting cord below. On breathing a 10 p.c. carbon dioxide mixture there was a normal response in the fingers similar to that described above, but there was no corresponding increase or decrease in the toe volume. In both subjects, however, coincident with a

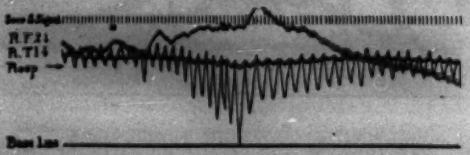

Fig. 5. *Paraplegia.* Inhalation of a mixture of 20 p.c. carbon dioxide in oxygen. Vessels initially dilated. Records from right second finger and right great toe. Signals—carbon dioxide mixture on and off.

decrease in the volume of the finger there was a slight increase in toe volume, without any corresponding increase in pulse wave. When the subsequent increase in finger volume occurred there was a slight fall in the volume of the toes (Fig. 5). It thus appears that the changes in digital volume seen in a normal subject are dependent upon the integrity of spinal pathways. In a patient with a completely denervated arm as the result of trauma to the brachial plexus, no change such as occurred in the normal limbs took place in the affected arm. This is evidence that the decrease and increase in digital volume are dependent upon the integrity of the peripheral nerves.

One subject who had had the left lumbar sympathetic chain removed was examined. During the inhalation of 20 p.c. carbon dioxide mixture a diminution in finger volume occurred. This was coincident with a decrease in the volume of the normally innervated toe. Simultaneously with this decrease in digital volume there was a slight increase in volume of the sympathectomized toe, without a corresponding increase in the size of the pulse wave. When increase of the volume of the normal digits occurred,

and as the blood pressure fell, there was a slight decrease in volume of the passive sympathectomized toe (Fig. 6).

Lastly, a subject whose preganglionic sympathetic fibres to the legs were intact, but who had complete section of the anterior and posterior lumbar and sacral nerve roots due to trauma to his cauda equina was examined. Responses similar to and simultaneous with those observed in the hands were produced in the toes on breathing 10 and 20 p.c. carbon dioxide mixtures.

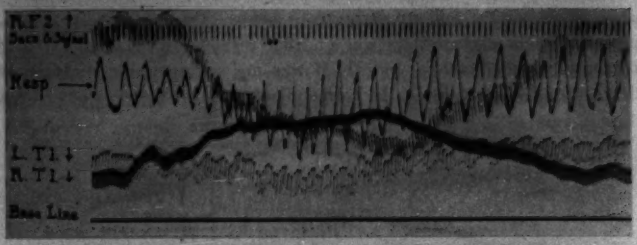

Fig. 6. *Left lumbar sympathectomy.* Inhalation of a mixture of 20 p.c. carbon dioxide in oxygen. Vessels initially dilated. Records taken from right second finger, and both great toes. Signals—CO_2 on and off.

It is thus evident that the changes produced by increase in carbon dioxide tension are dependent on the integrity of the sympathetic nervous supply to the digit. Conversely, no changes can be produced in denervated peripheral vessels by the direct effect of an increase in carbon dioxide tension in the perfusing blood to the degree used in these experiments. Again, as the volume changes described were absent in denervated limbs, they are independent of alterations in heart output, or of alterations in venous drainage and other mechanical factors which have been ascribed to the concomitant alteration in respiratory rhythm. Actually in the limb with denervated vessels such changes in volume as did occur were the converse of those observed in the normal digits. This further eliminates any peripheral influence of the carbon dioxide increase.

The changes which were seen in the denervated limbs were comparatively very small (Figs. 5 and 6). They were unaccompanied by a change in pulse wave. It is unlikely therefore that they were dependent on an alteration in cardiac output. They were always the reverse of the changes in the active limbs, with which however they were synchronous. It is probable that they were an expression of the passive response of the

denervated vessels to alterations in the available blood volume brought about by the changes in the normally innervated cardiovascular system.

B. Decrease in carbon dioxide tension produced no constant change in the digital volume either with the vessels in the dilated or constricted state. This was so even after periods of hyperventilation lasting for 15–20 min. After prolonged hyperventilation there was slight tachycardia accompanied by a decrease in the pulse volume. The decrease in pulse volume which occurred did not affect the digital volume proportionally.

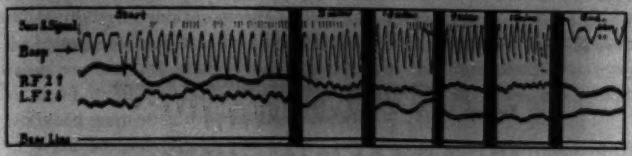

Fig. 7. *Normal.* Hyperventilation for 15 min. Vessels ntially constricted. Portion of a continuous record showing the finger volume at the commencement, after 2, 6, 9, 13 min and immediately after hyperventilation.

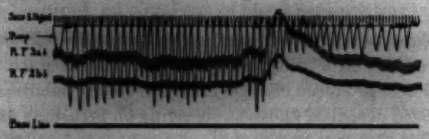

Fig. 8. *Normal.* Hyperventilation. Vessels initially dilated. Two records from the right second finger. Signals, hyperventilation commenced and ceased. At the signal for cessation of hyperventilation a deeper inspiration was made.

The changes were gradual and even after 15 min. the finger volume showed little change (Fig. 7). If hyperventilation were forced and unduly deep inspirations were made, inspiratory vaso-constrictor responses, similar to those described previously, occurred (Fig. 8). No constant alterations were observed in the blood pressure during hyperventilation.

C. Increase in oxygen tension caused no alteration in digital volume. The pulse wave and rate were unaffected by prolonged inhalation of pure oxygen, with the vessels in either an initially constricted or a dilated state.

D. Decrease in oxygen tension. Inhalation of pure nitrogen was maintained for periods up to 2 min. The subject became cyanosed and afterwards complained of a blurring of consciousness. There was a slight increase in the depth and rate of respiration. It was never so evident as that

produced by carbon dioxide excess. It was the rule to obtain no alteration in the digital volume. The pulse wave in the digit was also unchanged (Fig. 9). This was so even after a considerable degree of cyanosis had been produced. In a few cases a transient diminution in digit volume occurred, but repetition of the experiment failed to evoke it. This was not of the same character as that seen during inspiration of carbon dioxide mixtures. The increment was more rapid, and it was not followed by the profound increase in digit volume which was constantly observed in the latter case. It resembled rather the vaso-constrictor response to peripheral stimulation which has been described elsewhere. The effects obtained by rebreathing over KOH were similar to those produced by breathing nitrogen.

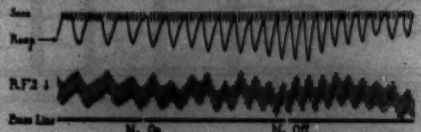

Fig. 9. *Normal.* Inhalation of pure nitrogen. Vessels initially dilated.

E. Reduction in oxygen with increase in carbon dioxide tension. With the vessels initially in a constricted and in a dilated state, rebreathing in a closed system produced changes in digital volume identical with those which have been described above as occurring on breathing excess carbon dioxide (Figs. 1–3).

Discussion

In this series of experiments an attempt has been made to study the reactions of the peripheral arteries and arterioles by plethysmography. Changes which are observed in the digital volume, in the absence of passive congestion or movements of the digits, may be due to changes in the capacity of the digital vessels, which may result from alterations in heart output, venous pressure or vasotonus. It has been shown that the changes described were absent in the affected limbs of subjects with a transverse dorsal cord lesion, and a brachial plexus lesion. They are therefore dependent on an intact nervous system and are independent of cardiac output. Further, they have been shown to be absent in a sympathectomized limb, but present in a limb to which the sympathetic nerves are intact, but in which the somatic nerves are severed. They are therefore dependent on the integrity of the sympathetic nervous system.

As venous congestion was excluded by the technique the change must therefore be the result of active vaso-motor changes in the digit. Conversely, constant finger volume demonstrates absence of vaso-motor changes.

That the methods used in these experiments for the production of changes in the composition of alveolar air produced adequate variations in the partial pressure of carbon dioxide and oxygen in the blood has been shown by Grollman [1930] and others. In these present experiments the subjects invariably described subjective symptoms after records had been taken, and in many cases marked distress or cyanosis was evident before the system was opened to air. It was, therefore, considered that the blood gas changes in these experiments were adequate. On the other hand, as complete unconsciousness or other untoward results were not obtained it was considered that the alterations in blood gas tension produced were within physiological limits.

With an increase in carbon dioxide tension there was a vaso-constriction when the subjects' vessels were previously dilated. This vaso-constriction was accompanied by an increase in systolic pressure. When the peripheral vessels were in a constricted state, although no further constriction occurred, an equal rise of blood pressure was registered. The rise was usually about 30–40 mm. Hg. It appears, then, that some other vascular bed—that of the muscles or splanchnic area—is primarily responsible for the rise in blood pressure. The subsequent rapid and marked vaso-dilatation that occurs commences after there has been a significant rise in blood pressure. It is closely followed in all cases by a fall in blood pressure to its initial level. The constant time relation between the vaso-dilatation and fall in blood pressure, which is independent of whether the carbon dioxide tension in the blood is rising or falling, suggests that the vaso-dilatation is a direct result of the rise in blood pressure. Again, as the maximum dilatation precedes the restoration of normal blood pressure, it is suggested that the vaso-dilatation is a compensatory mechanism, depending on the rapid rise in blood pressure and not on an alteration in the gaseous tension of the blood, as is the previous vaso-constriction and rise in blood pressure.

The denervated vessels of a sympathectomized limb have been shown to respond slightly in an inverse way to the normal limbs. It has been shown above that the increase in the volume of the sympathectomized digit, simultaneous with general peripheral vaso-constriction and rise in blood pressure, is probably due to the denervated vessels being stretched passively in response to the increased pressure—that is they have behaved as a reservoir for the excess blood. The normally innervated limbs,

however, have changed in response to a central mechanism. The evidence obtained therefore suggests that the alteration in vasotonus is not dependent on a peripheral metabolite effect of altered carbon dioxide or oxygen tension.

The literature on the subject under discussion is immense. It is also very conflicting. The primary cause for this conflict is the discrepancy between results obtained with the use of anæsthetized animals and those obtained in man. Grollman [1932] summed up the position by saying that "It is quite unjustifiable to draw broad generalizations concerning the role of carbon dioxide as a regulator of the circulation on the basis of anæsthetized, dismembered animals or on isolated strips of blood vessels, as has so often been done."

Dale & Evans [1922] have obtained a fall in arterial pressure on hyperventilating anæsthetized cats. Henderson [1918] has obtained similar results in dogs. Collier *et al.* [1927] were unable to find any alterations in blood pressure in man during voluntary overbreathing. This they have shown to be due to a compensatory peripheral vaso-constriction which is absent in anæsthetized cats and dogs. They stated that the constriction was due to respiratory effort. It has recently been shown that there is a vaso-constrictor response in man to deep respiration [Bolton *et al.* 1936]. If hyperventilation is continued below the threshold of this response for periods up to 15 min., no change in blood pressure or in the calibre of the peripheral vascular bed occurs in man. When, however, a deeper inspiration is made temporary vaso-constriction is brought about (Fig. 8).

Dale, Evans, Henderson and others have suggested that the changes seen in animals are largely independent of the cardiac output. As it has been shown in these present experiments that such changes as are produced by alterations in blood gas tension are absent in sympathectomized vessels, it is evident that in man also cardiac factors are negligible. Similarly the mechanical effect of hyperventilation produced by voluntary overbreathing of air or 5 p.c. carbon dioxide described by Vincent & Thomson [1928] can have played little part on the results obtained, since this effect would have been very evident in a passive denervated vessel.

Again the direct effect of carbon dioxide on the vessels which Henderson & Harvey [1918] described as a venous pressor mechanism in the cat can have had little part in the fall in digital volume in man, for a slight increase occurred in the passive vessels when a decrease occurred in the normal vessels.

Rehberg and Krogh [1929] have shown that cyanosis is accompanied by arterial and capillary dilatation but they were unable to state if this was central in origin. Lewis [1927] thought that the dilatation which he observed in the peripheral vessels following increase in the acidity of the blood was probably of central origin. Krogh [1918] produced great capillary dilatation in the frog's tongue by direct application of pure carbon dioxide. From the evidence produced above it would appear that any peripheral effect of alteration in carbon dioxide tension within the limits of these experiments does not produce a significant change in the capillary bed in a normal man.

Lastly, with regard to the experiments on man the literature is inconsistent. Not only have different authorities obtained different results, but workers have found widely varying results in a series of experiments. This was the experience of Schneider [1929] who investigated the arterial and venous blood pressure, pulse rate, hand volume and rate of blood flow during hyperventilation. It is probable that many of the differences which have been observed were produced by differences in the depth of hyperventilation and by individual differences in the initial state of the vaso-motor system.

Summary

In man alterations in the blood gas tensions have been produced. An increase in carbon dioxide tension in the blood causes a peripheral vaso-constriction, followed by a profound vaso-dilatation. The vaso-constriction only occurs if the subject's blood vessels are initially dilated, and has a fairly constant time relation to the inspiration of the carbon dioxide mixture. A rise in arterial blood pressure occurs simultaneously with the peripheral vaso-constriction. It is suggested that this rise is mainly dependent upon another vaso-motor mechanism, which is probably muscular or splanchnic.

The vaso-dilatation has no time relationship to the inspiration of excess carbon dioxide, but always precedes the fall in systemic blood pressure. It appears to depend for its initiation in a rapid rise in blood pressure, and is probably a compensatory mechanism designed to re-adjust the blood pressure.

Both the vaso-constriction due to increased carbon dioxide tension and the vaso-dilatation due to increased blood pressure are dependent on the integrity of the sympathetic nervous system and are under central nervous control.

No evidence in support of a direct peripheral vascular effect of alterations in blood gas tension has been produced.

All the responses elicited are vaso-motor in origin. Cardiac, capillary, and venous changes appear to be negligible within the limits of these experiments.

The only factor which *per se* produced these vaso-motor changes was an increase in the carbon dioxide tension in the blood.

We desire to thank the honorary staff of the National Hospital for permission to study patients under their care, and the post-graduates who offered themselves as normal controls.

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THE SPREAD OF ACTIVITY IN THE CEREBRAL CORTEX

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(*Received May* 29, 1936)

The electrical changes in the cerebral cortex should provide the most direct index of its activities. They seem to correspond with the electric changes in muscle and nerve, for they have the same rhythmic character and they are present whenever the brain is known to be active. There are, no doubt, important events in the neurones which have no electrical counterpart, but we can reasonably infer a wave of excitation when we record a potential wave on the surface of the brain.

In theory, therefore, it should be possible to learn what is happening in any part of the brain by recording the potential changes in that part. There is no difficulty in recording them, for oscillations of potential take place almost continuously between any two points on the surface of the cortex even though the animal is deeply anæsthetized. But unfortunately such electrical records from the brain are not yet of much value, for we lack most of the data needed to interpret them. We do not know what kind of cortical activity is revealed by a given potential change, for we do not know what kind of activity is possible, what structures are responsible for the potential changes, or how excitations spread from one neurone to another. The main object of the present experiments was to investigate questions of this kind. They have been dealt with recently by several methods: for instance, Dusser de Barenne [1934] has studied the effects of thermocoagulation of the different layers of the cortex, Bartley & Bishop [1933] have recorded the potential changes in the striate area when the optic nerve is stimulated electrically, and there have been various investigations of the effects of sensory stimulation and convulsant drugs on the cortical potentials. For the most part, however, our knowledge of the reactions of cortical cells has come from observations on the motor area stimulated electrically. In this region cortical

activity leads to immediate bodily changes. With these as indicator the motor area has played a decisive part in shaping our ideas of cerebral localization, and its reactions have also shown certain features which seem to express the distinctive behaviour of the neurones in the cortex. Such are the "facilitation" or "deviation" of the response [Graham Brown, 1916; Leyton & Sherrington, 1917], both showing how much the response depends on the previous history of the stimulated point; other characteristic features are the variable time relations of the movements and the development of convulsions with strong stimuli.

In the present experiments the brain has been stimulated electrically, and its activity has been recorded not by the movements of limbs but by the action potentials developed at its surface. The work is the natural outcome of that of Adrian & Matthews [1934] in which the cortex was stimulated by local injury. This gave a characteristic sequence of potential waves from which the time relations of the neurones could be inferred, but it was clear that more could be learnt from a less drastic stimulus. With electrical stimulation the cortical activity is under better control: the phenomena of facilitation, etc., can be studied and the potential change provides an index of activity which is more direct than the motor response and is available in any region of the cortex.

The final aim of the investigation was to learn more about the cell reactions which take place normally in the brain and about the relation between them and the potential changes which may be observed. For this the method has one serious drawback, inherent in the use of electrical stimulation on the brain of an anæsthetized animal. The activities produced in these conditions are easily controlled, but they are artificial and may have little relation with those which occur in normal life. For experiments on the motor area there have been two lines of defence, (*a*) that the activity evoked by the stimulus results in something like a normal movement, and (*b*) that the conclusions to be drawn from stimulation experiments agree with those from entirely different lines of evidence, clinical, histological, etc. In most of the present experiments the first defence cannot be urged, for the only sign of cerebral activity is the movement of an oscillograph mirror. But in some of them the motor area has been stimulated and the cortical response has been recorded simultaneously with the limb movements and these have shown a satisfactory correlation. For the rest it may be said that the activities revealed by electrical stimulation are not so very different from what we might expect of the normal cortical mechanism, and that at the least they show what may happen in pathological states.

One great advantage is gained by the artificial conditions of the experiment, namely, a reasonable agreement in the behaviour of different preparations. The anæsthetic prevents disturbance of the cortex by afferent impulses, and the electric stimulus forces a large group of neurones to discharge simultaneously. The result is a response of characteristic form which is modified by facilitation, etc., in a characteristic manner. In fact, the cortical reactions can be predicted with some accuracy as soon as a test stimulation has shown whether facilitation is likely to occur or not.

METHOD

(1) *Preparation.* The observations have been made on rabbits, cats and monkeys. The animals were anæsthetized, usually with dial in large enough doses to prevent all but very slight movements on strong sensory stimulation. A few experiments have been made with urethane, chloroform and ether or ether alone. The left cerebral hemisphere is exposed and the animal is placed on a stand, prone, with its head supported by a metal bar held between the jaws. Rods carrying the stimulating and recording electrodes are fixed to uprights mounted on the stand, and the preparation is removed to the large copper incubator which acts as a shield from electrical disturbances. In this it remains in a warm, moist atmosphere throughout the experiment. The exposed surface of the brain is irrigated from time to time with hot Ringer and covered with moist cotton wool between observations.

(2) *Stimulating apparatus.* The stimulating device must be capable of delivering a series of shocks at frequencies ranging from 5 to 50 per sec. The strength of the shocks must be variable and the duration of each must be as brief as possible. This last requirement arises from the fact that the recording electrodes must often be placed very close to the stimulating on the surface of a large mass of tissue. With such an arrangement an appreciable fraction of the stimulating current is bound to flow through the tissue under the recording electrodes, causing large "stimulus artefacts" to appear. No difficulty should arise from these if each artefact has subsided before the corresponding action-potential wave appears, but if the artefact is still considerable the record becomes very difficult to interpret. For this reason the briefer the stimuli the better, provided that they can be made strong enough to stimulate the cortex and that they are not then so strong that they affect distant structures which are more sensitive to brief stimuli.

After various trials it was found that the best form of stimulus for the particular amplifying and recording system in use was the break shock

from an induction coil with a 0·01 μF. condenser in each lead from the secondary. No earth connexion is made to any part of the stimulating system, for this increases the artefact. The primary current (controlled by a variable resistance) is made and broken by a rotating contact breaker which also short-circuits the make shocks. It is driven by an electric gramophone motor and is usually arranged to deliver from 10 to 30 shocks per sec.

As a rule unipolar stimulation has been employed, the active electrode being a fine silver wire insulated with enamel except at the tip. This can be brought into contact with the cortex by a screw adjustment. The metal bar between the jaws acts as the indifferent electrode.

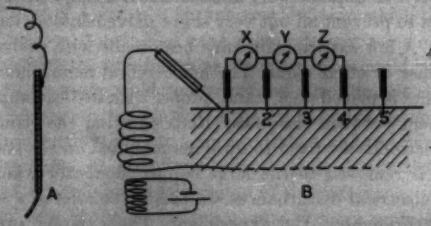

Fig. 1. A. Electrode used for recording from points close together on the brain surface. The silver tube is coated internally with silver chloride. B. Usual arrangement of stimulating circuit, electrodes and oscillographs.

(3) *Recording electrodes.* In the preliminary experiments only one pair of recording electrodes was used and these were of the usual Ag-$AgCl_2$-Ringer pattern made up in small glass tubes. Later on it became necessary to use several pairs of electrodes arranged very close together, and for this a specially compact form had to be devised. The chief requirements are that the lower end of the electrode should not be hard enough to damage the cortex and that the system should be sufficiently non-polarizable to record the potentials without distortion. The pattern finally chosen is illustrated in Fig. 1. Each electrode consists of a length (2 cm.) of silver tube, 1 mm. in diameter, with a silk thread filling up the bore and projecting about 3 mm. from the lower end. The thread is kept moist with Ringer's fluid during an experiment and the electrodes are stored in the fluid. From time to time a layer of $AgCl_2$ is deposited electrolytically in the interior of the tube by a current led through the thread. These electrodes are fixed with plasticine in grooves cut in an ebonite block which is carried on an adjustable arm. Two

blocks have been in common use, one to hold six electrodes arranged in line at intervals of 2·5 mm., and one for two lines at right angles. Smaller separations are given by moving the ends of the threads closer together on the cortex.

(4) *Amplifying and recording system.* In most of the experiments three Matthews' oscillographs were arranged to record simultaneously on bromide paper. All three can be driven by condenser-coupled amplifiers with a balanced input stage [Matthews, 1934], but as a rule only two of these amplifiers are used and the remaining oscillograph is driven by one with battery coupling. This makes it possible not only to record very slow changes of potential, but to investigate the region very close to the stimulating electrode, for a battery-coupled system takes an exceedingly short time to recover its balance after a large stimulus escape. No great sensitivity is required, for the potential changes to be recorded are of the order of 0·1–1·0 mV.

The usual arrangement of electrodes and amplifiers is shown in Fig. 1 B. Lead 1 nearest the stimulating electrode is earthed, lead 2 goes to the input of the battery-coupled amplifier (oscillograph *X*), the balanced amplifier for oscillograph *Y* is connected to leads 2 and 3 and that for oscillograph *Z* to leads 3 and 4. A downward deflexion on the record for any pair of leads indicates that the lead nearer to the stimulating electrode has become positive to points farther away. Controls were made from time to time to ensure that the three recording systems were not influencing one another, and that the position of the earthed lead did not influence the potential gradients.

Records were made on bromide paper moving up to speeds of $\frac{1}{2}$ metre per sec., and higher speeds were given by a rotating mirror camera. The deflexions were viewed in a rotating mirror: a loud-speaker system, though available, was seldom used.

Results

Except where otherwise stated the following account will deal with the potential changes in the cortex of the rabbit. In this animal the anæsthetic seems to produce less depression of excitability than in the cat or monkey, a spread of excitation occurs with greater regularity and can be analysed more easily, owing, partly, to the absence of sulci in the brain surface. Experiments on the motor area, correlating the muscular response with the cortical potentials, have been confined to monkeys and will be dealt with in a later section.

Electric response of the cortex to repeated stimulation

The typical effect of a series of electric stimuli to the surface of the brain is shown in Fig. 2. In these experiments only one oscillograph is in use and the recording electrodes are at 2 and 5–9 mm. from the stimulated point. The stimuli are break shocks with a strength about twice that necessary to give a contraction of the temporal muscle. The stimulus artefact at each shock makes a rapid excursion which appears in the record as a thin vertical line.

Fig. 2. Progressive increase in the electric response of the cortex with repeated stimulation. A. Cat. Stimulation 13 per sec. Successive portions of one record. Time mark gives 0·1 sec. B. Rabbit. Stimulation 28 per sec. Response at half-frequency in the third strip. Time mark gives 0·1 sec. C. Rabbit. Stimulation 17 per sec. In the second strip the stimulation is discontinued at the arrow. The after-discharge is made up of waves of the same type. Time mark (horizontal line) gives 1·0 sec.

In the records of the early stages of stimulation there is a small downward deflexion following each shock. These become larger and larger as the stimulation proceeds. They may change in form, developing subsidiary crests and troughs, and at a later stage the responses may be no longer all alike, larger deflexions appearing at every second or third stimulus (Fig. 2 B). Eventually they may become completely irregular and may fail, but if the stimulation is stopped whilst the deflexions are still very large there is usually an after-discharge as in Fig. 2 C.

That there should be this progressive increase in the electric response is a result in harmony with the progressive increase in the muscular response when the motor area is stimulated. Evidently "facilitation" is

not a phenomenon confined entirely to the motor response. The chief condition necessary for facilitation of the electric response is that the blood supply to the cortex should be adequate. If the smaller vessels are well filled and the cortical surface has a pink or reddish colour the increase is likely to appear, but if the surface is pale there may be an initial increase in the response followed by early failure, or there may be no increase at all.

Records showing an evolution of the response like that in Fig. 2 have been obtained from widely separated areas of the rabbit's cortex, and from rabbits anæsthetized with urethane, chlorestone and chloroform and ether, as well as with dial. They have been obtained from cats and monkeys, as well as from rabbits, though stronger stimuli and higher frequencies may be needed. It is therefore a characteristic property of the cortex to give an increasing electric response to repeated stimuli.

The immediate cause of the increase might be a more intense activity confined to the same group of neurones, or a spread of the excitation to a larger group. It was to decide such points that the potential gradients in the active region were mapped out with the three-oscillograph system. The results of this analysis fall into two main sections, the first dealing with the cortical response in its simplest form, and the second with the changes which take place on repeated stimulation.

The superficial response

If the stimuli are weak or of low frequency (below 8 per sec.), or if the animal is very deeply anæsthetized, the response does not alter in any way as stimulation proceeds. It is then confined to the neighbourhood of the stimulating electrodes and is usually a monophasic potential change lasting about 0·01 sec. and reaching a maximum value of 0·1 mV. Typical records at high speed are given in Fig. 3; the exact form of the response varies with the state of the animal, the position of the electrodes, etc., but the time relations are always of the same order.

The potential changes which are recorded in the region of the stimulus are due partly to the stimulus itself and partly to the response of the cortical elements. When the leads are 2–3 mm. away the stimulus contributes no more than a very brief oscillation of potential which is over before the slower neurone response has begun. When the leads are nearer, the stimulus artefact is larger and contributes (*a*) a gradually declining tail which merges with the response and (*b*) a shift in the base-line due to hysteresis in the oscillograph. This direct effect of the stimulus can be roughly estimated by reversing the direction of the current from time to time (Fig. 4), or by making a record after the cortical response has been abolished by deprivation of blood supply, etc. In many records a sudden oscillation of potential is visible midway between two stimuli. This occurs at the moment when the current is made in the primary of the induction coil, though the short circuit in the secondary seems effective enough in preventing stimulation at "make".

The most interesting feature of the response is shown by a diagram of the potential gradients on the cortex (Fig. 5). The gradients have been worked out from simultaneous records taken from several pairs of electrodes, or from records made with one oscillograph and a pair of electrodes moved farther and farther away from the stimulated point. The result is always that, at the height of each response, the stimulated point is negative to points a few mm. away, but that these are positive to points still farther off.

In the diagram in Fig. 5 the potential at the stimulated point (1) is shown as negative to the average potential of the cortex, and that of

Fig. 3. Superficial response (rabbit). The response is the slower downward deflexion which follows the abrupt stimulus artefact. In this record downward movement indicates a fall of potential at the stimulated point. Time marker gives 0·0035 sec.

Fig. 4. Superficial response (rabbit); showing the effect of reversing the stimulating current. The stimulus artefact and the hysteresis effect are reversed but the cortical response is not. Time 0·1 sec.

point 2 as positive. This is the usual finding, but to show that the active region is in fact negative to distant regions it may be necessary to use a lead which almost touches the stimulating electrode. To show that the active region is negative to points a few mm. away does not require so close an adjustment.

There is little doubt that the potential distribution shown in Fig. 5 is due to the response of cortical elements which are very close to the stimulated point and are all directly excited by the stimulating current. Unless facilitation occurs to complicate matters the response is as constant as that of a nerve trunk and not much less resistant to adverse conditions. There is a definite threshold for the stimulus: with increasing strengths the response becomes larger, but the time relations and distribution of the potential gradients alter very little, and both are

independent of stimulus frequency up to rates as high as 35 per sec. Increasing the depth of anæsthesia may cause a rise in the threshold, but the response can still be elicited, unchanged in form, after respiration has been brought to a standstill. In the cat it has persisted for 5 min. after complete failure of the circulation. In the rabbit it is more sensitive and has sometimes been abolished by continued stimulation.

This constancy of the response makes it unlikely that any part of it is dependent on a spread of the excitation from one neurone to another. It must be due to elements concentrated near the point of the stimulating electrode, for the reversal of the potential gradient occurs at a fixed distance a few mm. away. It must be due to elements close to the surface of the cortex, for if the stimulating electrode is thrust 1·5–2 mm. into the grey matter the negative potential gradient between leads 1

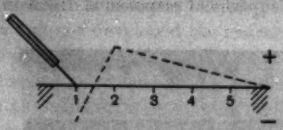

Fig. 5.

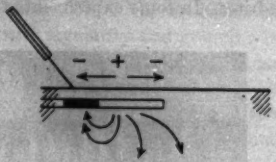

Fig. 6.

Fig. 5. Diagram to show potential gradients on the surface of the cortex during the superficial response.

Fig. 6. Probable disposition of active and inactive regions in the elements which produce the superficial response.

and 2 changes to a positive gradient. Again, if the surface layers are killed the negative gradient is no longer obtainable although the deeper layers can still respond. This was shown in several experiments in which Dusser de Barenne's thermocoagulation method was adopted, the cortex being touched for a few seconds by a metal rod heated to 65–70° C.

There is, of course, no difficulty in accounting for the fall of potential at the stimulated point. To establish any potential gradient there must be both a polarized (resting) and a depolarized (active) region in each neurone. The active region would naturally be found very close to the stimulating electrode, and an active region should be negative to an inactive. The rise of potential (relative to distant points) in the region a few mm. away is presumably due to the fact that the active elements are embedded in a medium which conducts electricity. The potential at the distant electrode will be the average potential of the whole element (nerve cell or fibre), whereas that at electrode 2 will be the potential of the

inactive part. This is positive to the active part and therefore positive to the average potential of the whole.

The diagram in Fig. 6 shows how the observed potential gradients might be produced by elements extending parallel to the surface and activated in the region of the stimulating electrode. These elements might well be nerve cells with dendrites spreading laterally, e.g. the horizontal cells of Cajal, or the superficial branches from the apical dendrites of pyramidal cells. It is unlikely that the response is due mainly to nerve fibres, for the potential changes are too slow to be the result of nerve-action potentials of the usual kind.

When the local or "superficial" response has the monophasic form shown in Fig. 4 there is presumably no complete shift of the active region from one part of the neurone to another, e.g. from the cell body to the dendrites. In some experiments the superficial response is diphasic and

Fig. 7. Spontaneous potential waves in cat's cortex occurring in groups with a frequency of about 10 per sec. A. No stimulation. B. Stimulation producing the superficial response. The waves are not affected. Time 0·5 sec.

the gradients shift during the course of each wave as though the active region were travelling outwards to the dendrite terminations. This has been found more often in the cat than in the rabbit. It may be related to a greater development of the superficial neurones in the cat than in the rabbit, for the reversal of the potential gradient occurs as much as 4 mm. from the stimulated point in the cat, whereas in the rabbit it is rarely more than 2 mm. away. The greater distance in the cat is only true of gradients measured along a gyrus: the potential changes beyond a sulcus are always exceedingly small. Deep $CHCl_3$ anæsthesia or prolonged and rapid stimulation may change a diphasic to a monophasic response, but apart from such drastic treatment the form does not vary. It is not yet certain whether it varies significantly from one part of the cortex to another.

Effect on neighbouring areas. The superficial response can be evoked without affecting what is happening in neighbouring parts of the cortex. In a cat which has been under dial anæsthesia for several hours there is

usually a spontaneous activity in the form of groups of oscillations with a frequency of about 10 per sec. separated by intervals of rest of 1–5 sec. These groups (which closely resemble the Berger waves in man) occur in any part of the cortex, but as a rule the discharge is not synchronized over areas more than a few mm. in diameter. If the cortex is stimulated whilst this kind of activity is in progress it is found that the occurrence of the superficial response does not produce any obvious change in the activity at points 1–2·5 mm. away (Fig. 7). The "spontaneous" groups of waves occur at much the same intervals and the waves have the same frequency and amplitude as before. The amplitude is small (0·1 mV.), and it is difficult to record very small changes close to the stimulating electrode; it is therefore impossible to say whether the groups of waves still occur there, but they occur unchanged in the region of the positive gradient which surrounds the negative area. When the spontaneous activity has not developed the characteristic regularity and grouping it is more difficult to decide whether stimulation changes it or not, but no clear instance of a change has been observed. The conclusion must be that in the anæsthetized animal the activity of superficial cells in the cortex is not necessarily transmitted to cells a few mm. away, or not transmitted effectively enough to alter their behaviour.

The deep response

To study the superficial response the conditions must be arranged so that no facilitation occurs. When the cortex is well supplied with blood and the strength and frequency of stimulation is adequate the responses soon change their character and begin to increase. The first sign of the change is the appearance of a positive instead of a negative potential in the stimulated region. Records illustrating this change are given in Fig. 8. There is at first the upward deflexion following each stimulus, indicating that the proximal lead is negative to the distal, but soon this gives place to a downward deflexion which becomes larger and larger as stimulation proceeds, reaching values of 0·5 mV. or more. What happens to the superficial response is not very clear; a small notch in the curve suggests that it is present as before and that its negative potential is masked by the larger positive component. But the main result is that with each stimulus points near the stimulating electrode now become initially positive to points farther away.

Though this response is sometimes monophasic and sometimes diphasic its time relations are characteristic, the initial spike usually lasting about 0·01 sec. as in the superficial response. The most reasonable

explanation of the change is to suppose that the predominant effect is now due to elements with an active region some distance below the surface and an inactive region nearer the surface (Fig. 9 A). Fig. 9 B illustrates this in terms of the neurones concerned. It is assumed that the stimulus now activates the bodies of the deeper pyramidal cells, the

Fig. 8. Change in the sign of the response with repeated stimulation. A. Rabbit. Stimulation 16 per sec. Time 1·0 sec. B. Monkey. Stimulation 12 per sec. Time 0·1 sec. Records at 2, 7 and 12 sec. from beginning of stimulation.

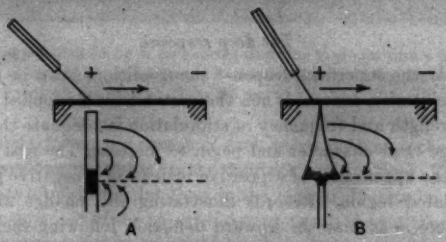

Fig. 9. Probable disposition of active and inactive regions in the elements which produce the deep response.

slender, apical part of the cell remaining (initially) inactive; thus the inactive apical surface would be positive to the active, basal surface, and on the cortex the region of the stimulating electrode would be positive to neighbouring regions. The lateral dendrites arising from the cell body are now left out of consideration; if they remain inactive their effect would counteract that of the apical process, but if they became active with the cell body they would increase the potential change at the surface of the cortex.

The view illustrated in Fig. 9 is supported by experiments in which the stimulating electrode is thrust into the cortex. If the change from the negative to the positive response is due to the activation of deeper structures, a stimulus which would affect those structures alone should give only the positive response. It has been stated already that it does so: if the tip of the electrode lies 1·5–2 mm. below the surface all trace of the negative gradient disappears and the initial phase of each response is a simple downward deflexion (cf. Fig. 10). The same kind of response is given when the superficial layers of the cortex are killed (see p. 135).

Fig. 10. Abolition of the superficial response by thermocoagulation of the outer layers. Rabbit. A. Normal, complex response with initial negativity at the stimulated point. B. Outer layers killed by contact with metal rod heated to 65° C. Deep response only. Time 0·1 sec.

When the stimulating electrode is on the surface of the cortex, the strength of shock required for the development of the "deep response" is usually twice or three times the threshold strength for the superficial response. This suggests that there must be some direct stimulation of the deeper layers and that they are not brought into play by impulses reaching them from above. At the beginning of the stimulation, however, there is usually very little indication of the deep response: thus with repeated stimulation either the deeper cells must become more and more excitable to an electric current or there must be a spread of activity from cell to cell over an increasing area.

The spread of the deep response

For the sake of clearness the change from superficial to deep response has been illustrated by records made with only one oscillograph. The subsequent increase in the deep response needs multiple recording to show its true character. Figs. 11 and 12 give records made with several pairs of leads at different distances from the stimulated point. When the increase is well established it can be seen at once that at each stimulus a wave of potential change spreads out from the stimulated area. Thus in Fig. 12 C the initial deflexions are downward, indicating that the proximal lead is positive to the distal: lead 1 has become strongly positive to lead 2

before any change is recorded between leads 2 and 3; lead 2 then becomes positive to lead 1 and lead 3; finally leads 1 and 2 become equipotential again and lead 3 becomes positive to leads 2 and 4. Thus the region of

Fig. 11. Development of conducted potential change moving outwards from the stimulated point. Rabbit. Stimulation 11 per sec. Electrodes at 0·5, 3, 5·5 and 8 mm. from the stimulated point (see inset in record C). A. 5 sec. from beginning of stimulation. Slow wave (spontaneous) in top tracing (oscillograph *Z*). B. 17 sec. Small monophasic response in *Z*. C. 27 sec. Response in *Z* now diphasic and larger. D. 45 sec. Slower conduction. Response in *Z* about 1·2 mV.

Fig. 12. Conducted potential change. A. Cat. Stimulating electrode 1·5 mm. in cortex. Electrodes, etc., as in Fig. 11. B. Rabbit. Beginning of stimulation. Deep response in *X*. Slow waves in *Y* and *Z* moving towards stimulus. C. After stimulation for 35 sec. Waves conducted to *Z*. Time 0·1 sec.

positive potential is rather sharply localized on the surface of the cortex and travels away from the stimulated point.

In the rabbit this outward spread of the response seems to be an invariable accompaniment of the increase with repeated stimuli. At

first it covers only a small area, reaching lead 2 (3 mm. from the stimulating electrode) though not lead 3 (5·5 mm.), but as stimulation proceeds each wave comes to travel farther and farther, reaching often as far as lead 4 (8 mm.) but rarely to lead 5 (10·5 mm.). The wave spreads as a widening circle. This is shown in Fig. 13, where two pairs of leads are arranged on radii at right angles with the stimulated region at the centre. On the average the waves reach the two proximal leads at the same moment and the maximum potential is developed at the same time in both pairs. Similar records have been made with two pairs of leads at either end of a diameter. A uniform spread of this kind is possible only in a cortex like that of the rabbit where there are no convolutions to distort the wave; but in the rabbit a region of local injury or a region where there has been prolonged stimulation may act as a barrier or may

Fig. 13. Spread of potential waves in a circle from the stimulated point. Rabbit. Electrodes arranged as in inset, along two radii at right angles, with 4 mm. between the stimulus and the proximal electrodes. The wave reaches both oscillographs at the same moment. Second record taken about 15 sec. after first. Time 0·1 sec.

start a spontaneous discharge. In fact, the wave front is seldom the perfect circle indicated in the diagram in Fig. 13.

The potential changes shown in these records must be due to waves of activity which spread out in the deeper layers of the cortex. The wave moves outwards because cell after cell develops a potential gradient between base and apex. Each wave travels a little farther than its predecessor, and so the whole response becomes larger and larger.

Repeated excitation. With rates of stimulation below about 20 per sec. there is often another change tending to increase the total response to each stimulus, for the simple mono- or diphasic deflexions become polyphasic, as shown in Fig. 14 A. The analysis of such responses is complicated by the fact that the active regions are not stationary and that each response may have components travelling different lengths from the starting point. The effects of conduction can be minimized by placing one lead on the cortex and one on indifferent tissue, and the response recorded in this way is often made up of two or sometimes three

deflexions of much the same form. The second deflexion sometimes rises and declines more slowly than the first and can be brought in or left out by altering the strength of the stimulus: it may then be due to the excitation of a different group of cells with slower time relations. As a rule, however, the second deflexion rises as rapidly as the first and the two crests are separated by an interval of about 0·02 sec. Presumably some of the cells are so strongly excited that they discharge twice to each stimulus. The interval between the two crests agrees with this, for Adrian & Matthews found that 50–100 per sec. was the maximum discharge rate for cortical neurones stimulated by injury.

Fig. 14. A. Development of polyphasic waves on repeated stimulation. Rabbit. B. Persistence of response near the stimulated point. Rabbit. The waves recorded in oscillograph *X* are triphasic, those on *Y* and *Z* are diphasic. Electrodes, etc., as in Fig. 11. Time 0·1 sec.

Fig. 14 B illustrates another way in which these polyphasic responses may be produced. There is the usual diphasic response between leads 2 and 3, and 3 and 4, but between 1 and 2 there are two downward deflexions with a momentary return to zero as lead 2 becomes positive to lead 3: thus the wave passes outwards leaving the stimulated region still active. The double response at lead 1 might be due to an unbroken period of activity in the neurones there or to a repeated excitation with a spread of the first but not of subsequent waves.

Frequency of deep responses. At the beginning of a period of stimulation following rest deep waves may be set up as often as 35 per sec., but if stimulation at this rate is maintained the frequency of the response drops sooner or later to a half or a third. Occasionally a wave is set up by every stimulus at the proximal pair of leads and only half of them reach the more distal. More often if there is a 2 : 1 relation anywhere it holds for the local as well as for the propagated response. An example

of the 2 : 1 response is given in Fig. 15. When the cortex is stimulated 15 times per sec. it responds at half this rate, whereas if stimulated at 12 per sec. it responds to every shock.

The limiting frequency for the conducted waves is lower than the 70–100 per sec. found by Adrian & Matthews for the maximum frequency of the local response to injury, but probably the recovery times are lengthened by the repeated excitation which is needed to produce conducted waves.

Fig. 15. Responses at half stimulation frequency. Rabbit. A. Stimulation 12 per sec. Response to every stimulus. B. Stimulation 15 per sec. Response to every other stimulus. The conduction is more rapid in B than in A owing to the longer interval between the waves. Electrodes etc., as in Fig. 11. Time 0·1 sec.

Conditions for facilitation. When each wave of activity travels a little farther than its predecessor we may say that facilitation is occurring. The conditions for facilitation are that the cortex should be well oxygenated and that the frequency of stimulation should exceed a critical value. In the rabbit it can occur with stimuli at 10 per sec., but it occurs more rapidly if the frequency is higher. In one experiment, for instance, stimulation at 10 per sec. had to be continued for 35 sec. to produce waves spreading 5 mm. from the origin, whereas with a frequency of 24 per sec. the waves could travel this distance after only 15 sec. stimulation. Deep anæsthesia seems to counteract the spread by producing a rapid fatigue; the deep response may appear and may begin to spread, but after a few seconds it becomes smaller and soon disappears, leaving only the superficial response (cf. Fig. 16). The same result is often found if the surface of the cortex is pale.

In the cat the spreading response can be obtained (cf. Fig. 12 A), but under dial the frequency of stimulation may need to be 20–30 per sec. A regular conduction of the waves through as much as 7·5 mm. has not been found, owing partly to a greater tendency for the series to be broken by large waves at irregular intervals. In the monkey's cortex a

frequency above 15 per sec. has been necessary to produce waves travelling as far as 3–5 mm., and again there is much more tendency to irregularities in the records. These differences will be considered in a later section.

Fig. 16. Rapid decline in response after initial increase in a rabbit deeply anæsthetized with urethane. Stimulation 19 per sec. Time 1·0 sec.

Fig. 17 *a*. To show the effect of a short pause on the degree of facilitation. Rabbit. A. Gradual increase of response at the beginning of stimulation. B. Rapid increase to former value after a pause of 2 sec. Time 1·0 sec.

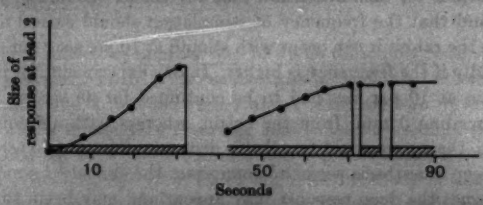

Fig. 17 *b*. Curve showing effect of pauses of 10 and 2 sec. on the size of the cortical response. Period of stimulation shown by shaded strip. The second pause produced a temporary increase in conduction velocity from 9 to 16 cm. per sec. Rabbit.

In the rabbit, according to the state of the cortex, the facilitation may build up slowly to decline rapidly when stimulation ceases, or may build up rapidly and may persist almost unchanged throughout a pause of 10–20 sec. The variations in the rate of development and persistence of the effect are illustrated in Figs. 17 *a*, *b*. In the latter the abscissæ

give the periods of stimulation and of rest and the ordinates the magnitude of the wave at lead 2. This is not necessarily an index of the distance travelled by the wave, for an increased potential at lead 2 implies an increased number of active neurones in that region, but this need not involve an extension of the area in which the activity occurs. As a rule, however, an increase in size is usually accompanied by a greater spread of the wave, and either size or spread might be taken as a measure of the facilitation.

The degree of facilitation attained after a given period of stimulation is probably governed by a number of interacting factors, but there are certainly two main opposing forces, the tendency for the activity to increase owing to the favouring effects of the previous wave and the tendency for it to decline from exhaustion or failure of the available energy in the cells. In some experiments the decline sets in so rapidly that it is never possible to reach the stage at which the wave travels as much as 2·5 mm.; in others a balance may be struck between the opposing tendencies, and for minutes on end the waves may travel 5 mm. but no more, in others the facilitation increases so much that the orderly conduction of the waves is soon interfered with by large outbursts from new foci.

Rate of conduction. The velocity at which the waves travel outwards can be estimated by measuring from the stimulus artefact to the beginning of the deflexion for each pair of leads. Thus in Fig. 18 A the times taken to reach leads 2, 3 and 4 are given by the three dotted lines. The velocity of conduction over a distance of 5 mm. from the origin is usually of the order of 25 cm. per sec. at some stage in the period of stimulation. It is impossible to give a more definite figure, for the velocity usually declines as the stimulation is continued.

The decline is illustrated in the curve in Fig. 18 B. Here the highest value is 60 cm. per sec. and the lowest 20 cm. At the beginning of the stimulation the wave may have travelled a shorter distance at a higher velocity, but in many experiments the velocity was never above 25–35 per sec., though the waves only travelled 2·5 mm. The lowest velocity recorded has been 5 cm. per sec.

A short pause in the stimulation produces an immediate rise in velocity, e.g. a pause of 1½ sec. increased it from 9 to 16 cm. per sec. It may be noted that a pause can change the velocity without necessarily affecting the degree of facilitation which is present: continued stimulation produces both slow conduction and conduction for a long distance, but a short pause may quicken the conduction without shortening the distance which the wave can travel.

Altering the frequency of the stimulation shows how closely the velocity is dependent on the interval between successive waves. This is illustrated in Figs. 15 and 19. In the latter the failure of a stimulus at W_4 allows the next wave W_5 to travel much faster. The frequencies

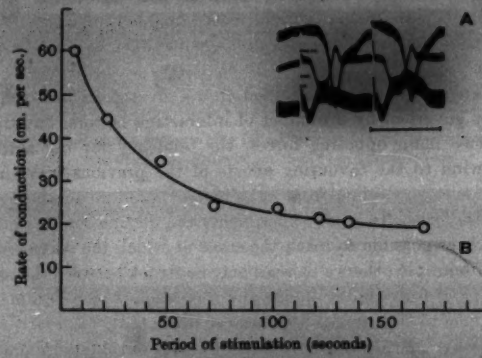

Fig. 18. A. Responses at leads 1–2, 2–3 and 3–4 after repeated stimulation, to show method of estimating conduction velocity. B. Decline in velocity with repeated stimulation. Rabbit. Stimulation at 11 per sec.

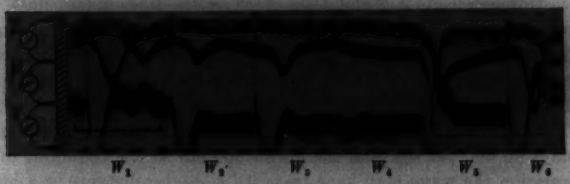

Fig. 19. Effect of a pause on the conduction velocity. The wave marked W_4 is very small and does not reach lead 2. W_5 reaches it in a much shorter time than W_4. Time 0·1 sec.

employed are not far from the limiting frequencies at which the cortex can respond, for in the experiment shown in Fig. 15, when the stimuli were increased from 12 to 15 per sec., every other one became ineffective. The rate of conduction then rises, since the interval between the waves is increased.

Evidently the waves travel slowly because the pathway is not allowed time for complete recovery and because the rate of recovery is diminished

as stimulation proceeds. The decline in velocity, like the increase in the distance travelled, comes on more rapidly at high frequencies of response than at low.

In some of the earlier experiments it was thought that the velocity of conduction diminished as the wave travelled farther away from the stimulated point, but later work has failed to show any constant diminution in velocity large enough to be outside the range of error.

Activity in neighbouring regions. The waves of activity which travel outwards from the stimulated point prevent the development of any other activity in the regions through which they pass. This is shown by the subsidence of all potential changes save those clearly related to the stimuli. In Fig. 12 B, for instance, at the beginning of stimulation there are large, slow fluctuations between leads 2, 3 and 4, but they cease as soon as the deep response waves reach these leads. On the other hand, regions not actually traversed by the waves seem to be quite unaffected by them. It is difficult to say how closely the two different kinds of activity may approach one another, but in one experiment the deep response waves reached lead 2 (2·5 mm.), whilst between leads 3 and 4 (5–7·5 mm.) there was a spontaneous oscillation at 12 per sec. In several experiments a search was made for a zone of inactivity surrounding the region covered by the deep response. None was found and no evidence of any modification in the behaviour of surrounding parts. Naturally the method could only show gross changes, for the spontaneous waves are too irregular to reveal minor disturbances.

After-discharges

After-discharges usually occur when the cortex is in good condition and has been stimulated rapidly enough to give an increasing degree of facilitation. They vary greatly in duration and magnitude. There may be no more than three or four potential waves travelling a few mm. from the stimulated point, or there may be repeated outbursts becoming larger and larger and ultimately involving the greater part of the cortical surface.

Such an after-discharge usually develops in a characteristic way: when the stimulation is over potential waves continue to arise from the region of the stimulating electrode. Lead 1 becomes positive to lead 2, lead 2 to lead 3, and so on, and the waves travel through the cortex for much the same distance and with much the same velocity as those which were set up by the stimulation (Fig. 20). Their frequency is often lower, but a regular series at 10–14 per sec. is not uncommon. Soon, however,

the waves grow larger and appear in groups with pauses in between; some of them start as before from the stimulated region, but many come from points 5 mm. or more away and travel towards instead of away from the stimulating electrode. Typical groups of waves are shown in Figs. 21,

Fig. 20. After-discharge waves resembling those produced by stimulation. Rabbits. A. The first three waves are due to stimuli. Of the five after-discharge waves all but the third start from lead 1 and reach lead 3 moving at much the same rate as the first three. B. During stimulation, and C, after-discharge in another rabbit. Oscillograph *X* (lower tracing) gives potential between leads 1 and 2, *Y* between 3 and 4. Time 0·1 sec.

Fig. 21. Development of grouped after-discharge. Rabbit. A. Regular waves starting from the stimulated point. B. Later. Groups, each beginning with a wave (W_1 and W_2) travelling towards the stimulated point. Electrodes etc. as in Fig. 11. Time 0·1 sec.

26 and 27. Each group represents a complex shifting of the potential gradients such as might occur from a to and fro movement of the focus of activity. The pattern changes very slightly from one group to the next but ultimately becomes simpler, and the pauses between the groups lengthen to $\frac{1}{2}$ sec. or more before the discharge ceases. During the latter part of the after-discharge electrical stimulation may produce regular waves as before, but as a rule it produces waves at a low frequency or in

groups. If the discharge develops very large potentials its cessation may leave the whole area inexcitable as well as inactive for 10–20 sec. After this the usual spontaneous oscillations return.

When the potential changes become very large it is probable that cells from every layer of the cortex contribute to them. This may alter the distribution of potential gradients about an active region and would explain certain of the complex grouped discharges where it is necessary to suppose either that there are several foci or that the active area may become negative. In records made with the direct-coupled amplifier there are often slow changes of potential between each wave or each group, particularly when the leads are near the place of origin of the discharge. They suggest a gradual change of polarization as a preliminary to each outburst, but it has not been possible to find them in every case.

Fig. 22. Waves occurring spontaneously during stimulation. W_1 and W_3 occur well before the corresponding stimulus. W_2 is just before, but W_4–W_8 are evidently set up by the stimuli. Between W_6 and W_7 a wave starts from lead 2. Electrodes, etc., as in Fig. 11. Time 0·1 sec.

The occurrence of an after-discharge can often be predicted before the stimulation is over. In Fig. 22, for instance, the waves seem to be partly determined by the stimuli and partly spontaneous: the excitability has been so raised that the neurones are ready to discharge of themselves if the stimulus does not anticipate the spontaneous outburst. Discharges coming from distant foci can also be found in some records during the stimulation. Fig. 23 is a good example. It was taken towards the end of a long period of stimulation and shows six waves, five starting from the neighbourhood of lead 1 and one from lead 3. The interesting feature of this record is that the waves W_1, W_2 and W_3 from lead 1 seem to travel with more and more difficulty. W_1 reaches lead 4; W_2 may do so, since the record from leads 3 and 4 is not a pure monophasic deflexion, but it travels more slowly than W_1; W_3 never reaches lead 3 at all, and then at the fourth stimulus a wave, W_4, starts from the region of lead 3 and travels outwards in both directions. Evidently in the region of lead 3 the cortex is less capable of responding at the

frequency of stimulation: successive waves are conducted through it more and more slowly and eventually find it completely refractory. Thus at the next stimulus recovery will be much further advanced at and beyond lead 3 than in the pathway up to it, and as the neurones are not far from the condition of spontaneous discharge, the slight effect of the stimulus at lead 3 will be enough to start the wave from there. The sequence shown in the figure was repeated many times, the wave starting from lead 3 at every fifth or sixth stimulus. A succession of events of the same kind is often seen in a true after-discharge though the sequence is often more complex (cf. Fig. 21 B).

Frequent changes of focus are to be expected in an after-discharge where the whole pathway has an increased responsiveness and where

Fig. 23. A wave starting from a distant point which has had a longer time to recover. W_2 reaches lead 2 but not lead 3. At the next stimulus the wave (W_4) starts from lead 3 and moves towards 1. The later waves start as usual from 1. Time 0·1 sec.

there is no longer an extraneous stimulus to start the waves from a fixed point. Owing to the repeated activity the neurones seem to have been brought into a condition in which they are closely linked and are all ready to beat spontaneously. To begin with, the pace is likely to be set by those near the stimulating electrode, for these have been the longest in action and so have had the best chance of developing the facilitated condition, but if they begin to work either too rapidly or too slowly, a wave will start from some other focus and for a time this will become the pacemaker. In fact, the conditions which may cause a shift in the focus of an after-discharge are not at all unlike those which may cause a shift in the pacemaker of the heart when the sino-auricular node is no longer predominant.

In magnitude, grouping, shifting focus, etc., the potential waves of an after-discharge resemble those produced by various convulsant drugs (e.g. thujone, picrotoxin, etc.). It will be shown later that they are closely related to the clonic epileptiform movements which may follow a

series of stimuli. In general terms they may be regarded as a natural result of the process of facilitation, though this statement can have little meaning until the nature of facilitation has been more thoroughly discussed.

Cortical responses in cats and monkeys

Although an outward spread from the stimulating electrode undoubtedly occurs in cats and monkeys, it cannot be demonstrated with the same certainty as in the rabbit. In cats this is partly due to a lower excitability of the cortex under dial anæsthesia, for as a rule with an electrode on the surface much stronger stimuli are needed to give an after-discharge. In monkeys after-discharges occur readily enough and there is the usual change from the superficial (negative) to the deep (positive) type of response and the progressive increase of the latter, but the deflexions are often polyphasic and so more difficult to analyse. This complexity is met with in cats as well as in monkeys and seems to be due to the more prominent part taken by the superficial elements in the cortex. The superficial response involves more widespread potential gradients than in the rabbit and is never wholly obliterated by the deep response. These complexities disappear, and the spread of the deep response is more easily followed when the stimulating electrode is driven 1·5–2 mm. into the cortex (cf. Fig. 12 A).

Another factor which hinders the development of a regular series of waves is the tendency for some to start at a distance from the stimulating electrode. With a superficial electrode the current will not be at all sharply localized in a cortical layer 2 mm. below the surface, but the fact remains that in the rabbit a long series of waves starts from some region very close to the electrode before extraneous waves begin: in cats and monkeys, on the other hand, they have appeared almost as soon as the deep response is established. In fact, it seems as though the waves cannot spread so regularly because the region they must traverse behaves far less as a uniformly reacting system: instead there are local inequalities of excitability which produce secondary foci before the facilitation has covered a wide area.

No systematic attempt has been made to find characteristic differences in behaviour in different parts of the cortex. These have been demonstrated for less artificially aroused activity by Kornmüller [1933, 1935] but in the present experiments none has been observed. The reactions to electric stimulation must depend so much on the fundamental properties of the neurone that the absence of gross local differences is not remarkable. In monkeys the time relations of the response, super-

ficial or deep, are not sensibly different for the frontal, temporal and parietal areas and for the pre- and postcentral gyri; but there is a wide variation at each point, since the responses are rarely simple monophasic waves.

Responses from the motor area

The motor area of the cortex offers an index of activity of an entirely different kind from that so far considered. Stimulating it produces movements of the limbs, and it is from these movements that most of our knowledge of cortical reactions is derived. It was obviously desirable, therefore, to compare the electric responses of the motor area with the movements produced by stimulating it. Eight *Rhesus* monkeys (average weight 3 kg.) were used for these experiments. All were anæsthetized with dial in doses of 0·4 c.c. per kg. The recording electrodes were arranged in a line on the left precentral gyrus, and one or more pairs of silver needle electrodes were driven through the skin into the muscles of the right arm or leg. As a rule two oscillographs recorded the cortical potentials and the third gave the electromyogram of the forearm, but sometimes records were made from two different muscle groups or from arm and leg.

The records show clearly that the superficial response is not associated with movement, but that the deep response is associated with it very closely. If the stimulation has not the necessary strength or frequency to cause the change from negative to positive deflexion no movements occur; movement begins as the positive deflexion increases in size, ceases if it declines again (as it may do in a deeply anæsthetized animal), and continues after the stimulation if there is a cortical after-discharge but not otherwise. The change from superficial to deep response and the increase in size of the latter proceed gradually, and it is not possible to say just how large it must be before a movement can be produced. Some degree of facilitation seems to be necessary, however, for in a deeply anæsthetized animal a stimulating electrode driven 2 mm. into the cortex will produce a localized "deep" response without arousing any movement.

The fact that movements do not begin until the stimulus has evoked an electric response in the deeper layers of the cortex agrees with Dusser de Barenne's finding that the three outer layers can be killed without affecting the motor reactions [Dusser de Barenne, 1934]. Records illustrating the parallel development of the movements and the cortical response are given in Figs. 24 and 25. Fig. 26 illustrates the very close correspondence in an after-discharge.

Fig. 24. Comparison of response from motor cortex (below) with action currents from arm muscles (above). Monkey. Dial. Stimulation 18 per sec. In record A the cortical response is of the superficial type (negative wave) and there is no arm movement. In B, a few seconds later, the cortical response shows a small positive (deep) component and there are action currents in the arm muscles following each stimulus. Time 0·1 sec.

Fig. 25. Records from motor cortex and forearm muscles. *X* records from leads 1 and 2, *Y* from 2 and 3, *Z* from the forearm. Stimulation 25 per sec. In A, at the beginning of stimulation the deep response is absent and there is no movement. In B, the deep response has appeared and each stimulus produces a group of action currents in the forelimb. In C, the action currents are nearly continuous. In D, they occur in groups corresponding to the cortical responses. Time 0·1 sec. Interval of 5 sec. between A and B and between B and C, 8 sec. between C and D.

Fig. 26. Comparison of cortical after-discharges produced by stimulating the motor area and action currents in arm muscles. In both records the muscle action currents are shown in oscillograph *Z* (top tracing). A. Close correspondence of large waves. B. Another monkey. Longer latency of movement. Time 0·1 sec. (Cortical responses from leads 1–2 and 2–3, all on arm area.)

Records from different parts of the motor area show that there is a spatial as well as a temporal correspondence: stimulation of the cortex will not give an arm movement unless it gives a response in the arm area, for a leg movement the leg area must be made to respond and so on, and this applies both to the period of stimulation and to that of the after-discharge. It so happened that none of the animals gave the generalized convulsions which sometimes follow strong stimulation, so that it has not been possible to demonstrate the widespread cortical responses which should accompany them. But the converse is shown in Fig. 28 which records a clonic movement restricted to one limb and a cortical after-discharge restricted to the one pair of leads.

Fig. 27. Correspondence between cortical after-discharge and movement. Arm area stimulated, clonic after-discharge movements in arm but not elsewhere. A. Lowest tracing (*X*) from leads 1–2 on arm area; middle tracing (*Y*) from leads 2–3 on arm area; top tracing (*Z*) from forearm muscles. B. Lowest tracing (*X*) from leads 1–2 on arm area; middle tracing (*Y*) from leads 4–5 on leg area; top tracing (*Z*) from forearm muscles. Time 0·1 sec.

Latency of motor response. Cooper & Denny-Brown [1927] have recorded the electric response of the monkey's arm muscles when the cortex is stimulated at different rates. The present results agree with theirs, though not covering so wide a range of frequencies. At the beginning of a movement there is a single action current volley in the muscle for each stimulus to the cortex, later a group of three or four, and later still a continuous succession with the stimulus rhythm no longer apparent (Fig. 25 C). At the beginning a latency of about 0·01 sec. intervenes between stimulus and muscle action potential, but this may be reduced to 0·005 sec. or even less when the groups take the place of the single response. In some records there has been a stage in which groups of waves still show the rhythm of stimulation but begin sometimes

a little after and sometimes a little before a particular stimulus. Presumably the cortical neurones are then giving occasional spontaneous beats as in Fig 22.

Various attempts have been made to influence the latency by stimulating at different points on the cortex, but its value changes so much as the response increases that it has not been possible to detect any local effect. A shift in the position of the stimulus may change the nature of

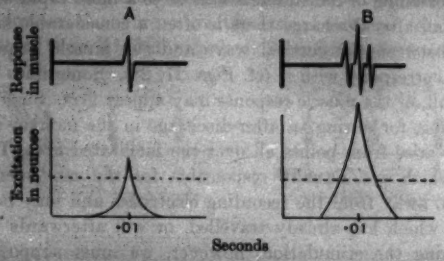

Fig. 28. To illustrate reduced latency of muscular response owing to increased intensity of excitation in the motor neurones. A. Early stage. B. Later, multiple responses in muscle.

Fig. 29. Stimulation of motor cortex (monkey). Action currents in biceps (upper tracing) and thumb muscles (lower), showing earlier response in biceps but coincidence of large waves in both muscles. Stimulation 28 per sec. Time 0·1 sec.

the movement, but if the forearm muscles still play a part in it, the range of latencies will not alter. The shortening of the latency as the response increases is due, as a rule, not to an earlier attainment of the maximum activity in the muscle, but to the activity developing progressively after each stimulus instead of abruptly. The response at the beginning of the stimulation period may be represented as in Fig. 28 A, and that at a later stage as in Fig. 28 B. The single and the multiple response can sometimes be recorded simultaneously by leading from two muscles, one of which is only brought into play when the movement is well developed. In Fig. 29, for instance, the upper record is from the biceps and the lower

from the thumb muscles. The arm began to contract some time before the thumb, and in the record the biceps response is multiple and has a latency of 0·007 sec. The thumb muscles give a single response with a latency of 0·010 sec., but it will be seen that the single waves of the lower record coincide with the largest waves in the upper. From such results it is reasonable to conclude that the shorter latency is due mainly to an increase in the intensity of the process which evokes the motor response, not to a shortening of its time relations or to a more rapid conduction.

During an after-discharge there is often a considerable latency (e.g. 0·03 sec.) between the cortical wave and the muscle response which appears to correspond with it (cf. Figs. 27, 28). Sometimes there is no latency at all, or the muscle response may appear first. Such results are not surprising, for during an after-discharge in the monkey's brain the waves may arise from points all over the facilitated area. The cortical neurones which are actually responsible for the movement may be several mm. away from the recording electrodes and may be activated by a wave which has already travelled, or will afterwards travel past them. During the stimulation, however, we must suppose that the neurones responsible for the movement are always close enough to the stimulus to be activated without appreciable delay, since the latency then is never very long.

Discussion

The artificial conditions of these experiments have been pointed out already. Electrical stimulation must produce an abnormal activity; the anæsthetic must have a depressing effect on the spread of activity through the cortex and may completely suppress certain modes of conduction. But the reactions illustrate properties of the cortical neurones which may play some part in the normal working of the brain and must certainly do so in pathological conditions.

It is clear in the first place that the potential waves are not an entirely artificial product but are a true index of the activity of cortical nerve cells. This follows from the close correspondence between the potential waves in the motor area and the activity of the limb muscles. What is, no doubt, abnormal is the degree to which the cortical neurones are forced to work in unison. In a smooth voluntary movement there is probably nothing like the synchronous activity occurring with electrical stimulation or in the after-discharge, and the potential changes may be much smaller and more confused. But the fact remains that whenever the neurones work synchronously the potential changes may be trusted to show what they are doing.

The potentials have one unexpected feature, that the activity of the deeper layers of the cortex makes the surface positive to neighbouring points instead of negative. Bartley & Bishop [1933] record a similar result in some of their work on the striate area. In the present experiments there have been occasional after-discharges in which the earliest surface change has been a fall of potential, but it has been a rise in all those in which the movement of the waves could be clearly traced. If the explanation given on p. 138 is correct the positive response means that the deeper parts of the neurone are depolarized at a time when the apical prolongation of the cell is still polarized. Thus the wave of activity which

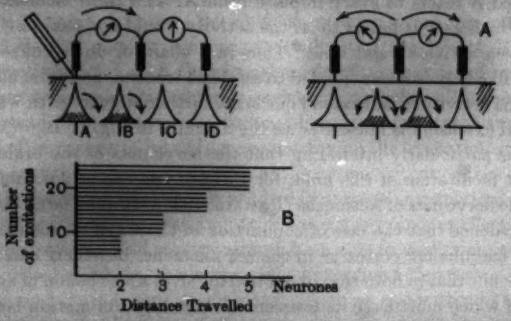

Fig. 30. A. To illustrate movement of potential wave owing to activation of a succession of neurones. B. To show gradual increase in distance travelled by wave if five excitations are necessary to establish conduction from one neurone to its neighbour.

spreads through the cortex involves the lower part of the cell body and the lateral dendrites (or either of them) before the apical part (Fig. 30). In fact, conduction takes place at a level corresponding to the basal rather than the apical part of the pyramidal cells—a conclusion which seems likely enough.

The movement of the potential waves is certainly due to a conduction of activity from neurone to neurone. This follows from the magnitude of the waves and particularly from the fact that they spread out in a circle without loss of amplitude. Impulses in nerve fibres radiating from the stimulated point and embedded in grey matter would give potential changes which would decline rapidly as the distance between recording and stimulating electrodes was increased, but the waves in the rabbit's cortex may be as large at 8 mm. from the stimulus as they are at 3 mm., and this is true for the whole circumference of the circle of

8 mm. radius. Thus the wave as it moves outwards must involve more and more neurones and its spread must be due to synaptic transmission from one neurone to another. The same conclusion has been reached by Bartley & Bishop [1933] for the potential waves produced by stimulating the optic nerve in the initial stages of strychnine poisoning.

The gradual increase in the distance travelled by successive waves implies no more than a simple type of summation at the synapses. There are now many examples of synaptic or neuro-muscular transmission in which the activity does not pass from *A* to *B* until *A* has been active several times in rapid succession, though once the transmission has occurred *B* reacts to every impulse from *A*. If a third element *C* were linked to *B* as *B* is linked to *A*, the activity would not pass to *C* until *B* had been active several times. Thus in a chain of units linked in this way if five impulses were needed to establish transmission from one to the next the activity would extend one stage further at every fifth wave. The gradual extension is illustrated in the diagram in Fig. 30 B.

It is particularly interesting that the nerve nets of the brain should exhibit facilitation of this kind, for Pantin has recently demonstrated it in the nerve nets of Actinozoa [Pantin, 1935]. It must be no more than a coincidence that the rate of conduction of the wave is much the same in the mammalian cortex as in the sea anemone, but there can be very little doubt that in both the conduction involves a succession of synapses, each of which must have its resistance lowered by summation before the wave can pass.

The process of summation, besides facilitating the passage of an impulse from one neurone to the next, raises the general level of excitability of the neurone and brings it nearer to the state in which it discharges spontaneously. This, at least, is the most reasonable explanation of the after-discharge. Those occurring in spinal reflexes have been ascribed to the late arrival of impulses set up during the period of stimulation and conducted by "delay paths" to the motor neurones [Forbes, 1921; Eccles & Sherrington, 1931]; but the delay path hypothesis, whatever its merits for reflex after-discharge, is scarcely applicable to waves which arise from the stimulated point itself or from somewhere very near it. A continued excitation at that point seems much more probable.

The subsequent course of the after-discharge suggests a conflict between the process by which each wave makes the system more unstable and an opposing process which causes a slower and slower recovery and ultimately stops all activity for many seconds. This process might be an inhibition, an accumulation of waste products or an exhaustion of active

material, though if it is an exhaustion it must be one which affects every neurone—even those which have only come into action at the height of the after-discharge.

The reactions involved in the spread of the wave and in the after-discharge are certainly responsible for the clonic movements which follow intense stimulation of the motor area. They are probably concerned in the production of convulsive movements by drugs such as thujone, camphor, etc., for the potential waves in the cortex are of the same type [Adrian & Matthews, 1934]. In fact, the spread by summation is clearly of importance in "experimental epilepsy" and, by extension, in all convulsions of cortical origin.

There remains, however, the obvious question: are these reactions ever called into play in the normal working of the brain? Has the anæsthetic interfered with the normal mode of conduction of impulses in the cortex, leaving one which is essentially pathological, namely the direct spread from a neurone to its neighbours in a facilitated area? The absence of any signs of inhibition accompanying the excitation suggests that the anæsthetic has cut off much of the normal activity of the cortex. Again, the reactions were usually studied in animals anæsthetized deeply enough to prevent sensory stimuli from having any obvious effect on the cortical potentials. Had sensory stimuli been able to modify the activity of the cortical neurones, the latter might have shown themselves able to influence one another over much longer distances and without the need for progressive facilitation of the pathway; indeed, the existence of association fibres implies that they can do so.

But in spite of the likelihood of easier conduction in the unanæsthetized cortex, it is not at all probable that the reactions considered in this paper will never take place, even to a minimal extent. The repeated excitation of a neurone, whether by electrical stimulation or by afferent impulses, could scarcely avoid facilitating the path between that neurone and its neighbours. No doubt in a normal cortex a repeated stimulation by afferent impulses would never produce activity over so great an area or a wave spreading uniformly in all directions. There would be regions of high and low excitability—due to previous activity—which would direct the wave along particular channels, and the whole effect would be on a much smaller scale.

In the present experiments the pathways formed by summation from one neurone to another are of the simplest character and have a life reckoned in seconds or at most in minutes after the stimulation has ceased. There is no evidence that they can ever become complex enough

or permanent enough to be the basis of a learnt reaction. The possibility of establishing pathways, or freely conducting areas in the cortex will not, of course, explain learning: there must be some means by which a whole pattern, temporal as well as spatial, of afferent excitations can arouse a pattern of motor activity. In the present experiments complex patterns of activity have often occurred in after-discharges, and these have tended to repeat themselves again and again. It has been shown how they may be formed as a result of slight differences in excitability, recovery time, etc., at different points in the area traversed by the waves. But such gross patterns, though they may be important in explaining pathological events like the stereotyped actions of epilepsy, can have very little relation to the neural changes involved in learning. All that can be said is that they reveal a behaviour which may be one of the essential parts of the learning process, though obviously it is not the whole of it. Further elaboration of the point is unnecessary, for the general problems of facilitation in relation to learning have been many times discussed.

Conclusions

1. In rabbits under dial anæsthesia electrical stimulation of the cerebral cortex gives rise to characteristic potential changes at its surface. These may be analysed by recording simultaneously from several pairs of electrodes.

2. The simplest response, evoked by weak stimuli, is a potential change lasting about 0·01 sec., in which the stimulated region becomes negative to points a few mm. away, though these are positive to points beyond. This response is due to neurones which are in the superficial layers of the cortex and are all directly excited by the stimulus.

3. Repeated stimulation with stronger currents brings about a change in the form of the response. The stimulated region now becomes positive to distant points, and the responses become larger and larger as stimulation proceeds. The chief potential change is now due to elements with the active region some distance below the surface.

4. When the deep response has developed, it is found that at each shock a wave of potential change spreads outwards in a circle from the stimulated point. Each wave travels a little farther than its predecessor, involving more neurones and so producing a greater response.

5. The movement of each potential wave over the cortex is due to a conduction of activity from neurone to neurone in the deeper layers. A summation of excitations is necessary before one neurone can transmit freely to its neighbours; hence the gradual increase in the distance travelled by successive waves.

6. In the rabbit's cortex the potential waves may travel 5–8 mm. from the stimulated point, at a velocity which declines with stimulation but is rarely more than 40 or less than 10 cm. per sec. The maximum frequency at which the waves can be set up declines progressively. The velocity of conduction increases if the interval between successive waves is suddenly lengthened.

7. In the regions through which the waves are conducted no other potential changes occur, but in regions just beyond there is no interference with the normal spontaneous activity.

8. In conditions which favour the spread of the waves the period of stimulation may be followed by an after-discharge. Initially the after-discharge waves start from the same point and follow the same course as those set up during the stimulation: later they may become grouped, the successive waves in the group starting from different points so that a complex pattern of activity results.

9. In the cat and monkey there is a greater tendency to irregular conduction and to waves starting from extraneous foci, but a spread can be demonstrated as in the rabbit.

10. When the motor area of the monkey is stimulated it is found that the appearance of movements in the limbs is correlated with the appearance of the "deep" cortical response. In after-discharges there is also a spatial and temporal correspondence between the potential waves in the cortex and the movements of the limbs, the stage of clonic movements corresponding to the grouped after-discharge.

11. The activity aroused by electrical stimulation in an anæsthetized animal is no doubt abnormal, but demonstrates reactions which probably occur in the normal brain.

The expenses of this work were defrayed by a grant from the Foulerton Committee of the Royal Society.

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ALKALINE RESISTANCE AND SPREADING VELOCITY OF FŒTAL AND ADULT TYPES OF MAMMALIAN HÆMOGLOBIN

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(*Received June* 4, 1936)

In a previous paper [Brinkman & Jonxis, 1935] in which the alkaline resistance and spreading velocity of human hæmoglobin was examined, we demonstrated the individuality of various kinds of hæmoglobin with this technique. We were not able, however, to find a distinction between any fœtal and adult hæmoglobin except that from man, although this might be expected from the researches of Barcroft and others on the dissociation curves of fœtal and maternal goat's hæmoglobin [Barcroft, 1935]. This was largely owing to technical difficulties in our method of determining the alkaline resistance of mammalian hæmoglobins (except for human blood), but we have now modified the method, so that any type can be examined accurately.

The result is that in the goat, cow and rabbit a distinction between fœtal and adult animal forms is clearly present. This statement is fully corroborated by the respective surface-pressure curves.

Method of making alkaline denaturation curves of animal hæmoglobin

About 1 c.c. of the blood is washed with isotonic saline in the centrifuge and a 40 vol. p.c. suspension of the corpuscles is made, which is hæmolysed by addition of some solid saponin. This is placed in a 37·0° C. bath, in which also a tube, containing 8 c.c. of the alkaline buffer solution is present. This buffer is a mixture of 0·15 *N* Na_2HPO_4 solution and *N* NaOH; its *p*H, and the *p*H after addition of the hæmoglobin solution and completion of denaturation are measured by means of the glass electrode, which could still be used in the range between *p*H 11 and 12.

A series of twenty 2 c.c. small centrifuge tubes was filled with 1 c.c. of a solution, composed of 500 c.c. saturated ammonium sulphate, 500 c.c. 0·15 *N* Na_2HPO_4 solution and 55 c.c. *N* KH_2PO_4 solution. This precipitates the denatured hæmoglobin, leaving the

undenatured in solution. 0·5 c.c. of the hæmoglobin solution to be examined is well mixed with 8 c.c. of the alkaline buffer solution at 37·0 and the stop-watch is started. Every minute (or every other minute) 0·5 c.c. of the denaturing solution is collected by means of a suitable syringe and injected into the 1 c.c. solution described above, which stops denaturation and precipitates the denatured product, with prevention of reversion to the native substance. After standing for 15 min. the tubes are centrifuged and the concentration of the not denatured hæmoglobin is determined.

The latter estimation must be made very carefully, as very small absolute concentrations are to be determined. We used a Hippel high-pressure mercury arc[1] and an orange filter which only transmits the 580 and 546 lines. Constancy of illumination is obtained by using a 100-watt generator, driven by a synchronous motor of 1500 rev. per min. A separate photo-electric cell (Weston), in series with a specially adapted pointer galvanometer, controls the stability and standard value of illumination, which can be corrected by a 400-ohm resistance in series with the mercury arc; the current taken is 150 mA.

The absorption by the solution examined is measured by a sensitive photo-electric "Sperrschichtcell" (Suddeutsche App. Fabrik, Nurnberg), the E.M.F. of which is measured by potentiometer. Calibration is made by making a dilution curve of the original Hb solution in each experiment; the dilution is made with the same salt solution in which the denatured hæmoglobin is finally present.

This method is very satisfactory, and accurate to about 0·1 p.c. of the original solution before denaturation. The surface-pressure curves were made with the technique described in our previous paper [Brinkman & Jonxis, 1935].

The results are given in Fig. 1 for goat's blood, which was taken because most of Barcroft's work was done with this species. The denaturation was made at *p*H 11·80, at 37·0° C., with a constant amount of hæmoglobin from washed corpuscles. It is seen that fœtal goat's hæmoglobin is distinguished by a reaction velocity which makes it much less alkali-resistant than the adult form. The blood 1 day after birth contains about 10 p.c. of the adult form, and this amount gradually increases, so that at the age of 4 weeks the percentage of hæmoglobin is about 30 p.c. The hæmoglobin of the adult and fœtal cow shows just the same difference in alkali resistance as the goat does. But fœtal rabbit's hæmoglobin is similar to the fœtal human pigment, its alkali resistance surpassing that of the adult form. In the cat no difference between very young and older hæmoglobin could be found. The results of spreading experiments lead to the same conclusion. The method is described in the previous paper, but a brief sketch may be inserted here.

A small amount of solution of protein, put carefully on a water surface, spreads to form a monomolecular film. The extent of this film depends on the kind of protein and the electrolyte properties of the carrier solution. On strong acid, base or salt solution the surface is at

[1] Schott u. gen., Jena.

once maximal, but on dilute buffer solutions the initial surface is much smaller and depends on the *p*H.

Only at the isoelectric point does the spreading become large or maximal again, and so the measurement of spreading velocity on dilute

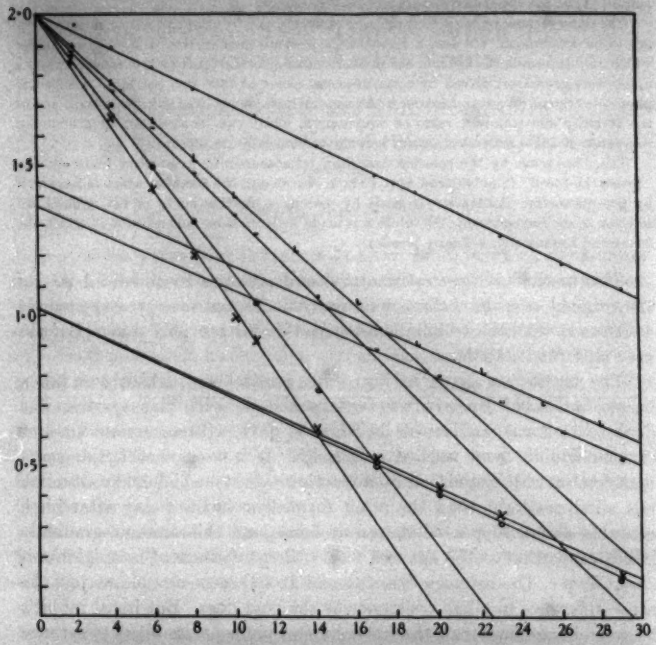

Fig. 1. Rate of alkali denaturation of adult and fœtal goat's hæmoglobin. ... adult hæmoglobin; × × × 2 days after birth; ooo 9 days after birth; : : : : 16 days after birth; LLLL 23 days after birth. Vertical axis: log percentage unchanged Hb. Horizontal axis: time in minutes.

buffer solutions of different *p*H is characteristic, and maximal in the isoelectric point of the protein examined. In Fig. 2 the spreading velocity curves of fœtal or adult hæmoglobins of various mammals are shown. The maximal surface is the same for all hæmoglobins, namely 1·15 square metres per mg. The spreading velocity at the isoelectric point is very

different however and it is characteristic of the species. In man, and in the rabbit, the isoelectric spreading velocity is larger in the adult, but smaller in the fœtal type. In the cow and the goat the situation is reversed, the fœtal type having the quicker spreading. The change from

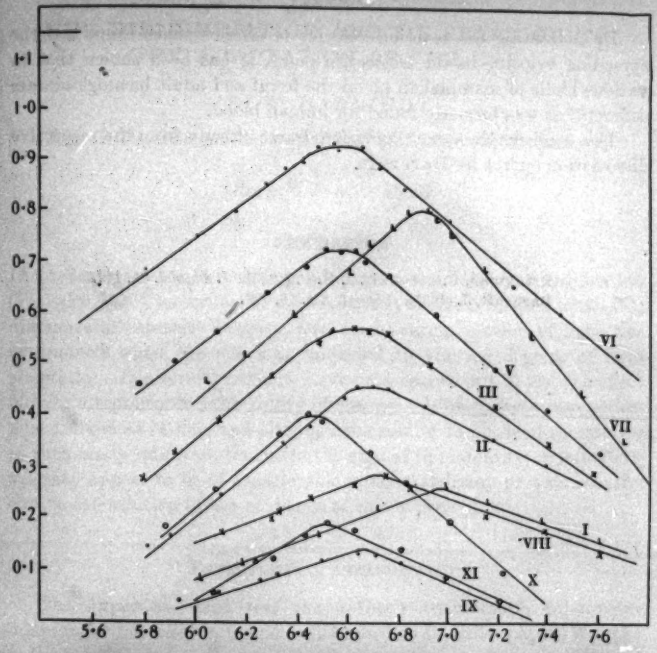

Fig. 2. Spreading of hæmoglobin of adult goat (I), fœtal goat (VI), 2 days old goat (V) 9 days old goat (IV), 16 days old goat (III), and 23 days old goat (II). Adult rabbit (VII), fœtal rabbit (VIII), cow (IX), newborn calf (X), and cat (XI). Vertical axis: surface of 1 mg. of hæmoglobin in square metres after 1 min. of spreading at 20° C. Horizontal axis: *p*H of buffer solutions.

the fœtal type to the adults can also be observed. In the cat no differences between fœtal and adult hæmoglobin can be seen.

It will be clear that these results correspond very well to the conclusions from denaturation curves, and further that a parallel may be drawn with the distinction of fœtal and adult oxygen dissociation curves.

In man the fœtal oxygen dissociation curve of pure hæmoglobin is to the right of the adult form, in the goat the relative positions are reversed, just as it is described now for the above mentioned properties.

SUMMARY

By measuring the reaction velocity of alkali denaturation and the spreading velocity in the isoelectric point, it has been shown that in various kinds of mammalian blood the fœtal and adult hæmoglobin are different, as was formerly found for human blood.

This confirms the same conclusion drawn already from the respective dissociation curves by Barcroft.

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HUE DISCRIMINATION AND ITS RELATION TO THE ADAPTATION OF THE EYE

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Introduction

In a recent paper on the effect of adaptation on intensity discrimination [Wright, 1935], experiments were described in which the just noticeable difference in intensity between two neighbouring patches of light was determined when the eye was subjected to varying degrees of light adaptation. Similar experiments have been carried out to test the effect of light adaptation on the ability of the eye to differentiate just noticeable differences in hue, and although the results do not lend themselves to such ready interpretation as in the case of the intensity experiments, yet they appear to be of considerable interest and may provide a useful clue to the solution of the problems of colour perception.

Experimental arrangements

The apparatus used was the author's trichromatic colorimeter adapted for hue discrimination as described in an earlier paper [Wright & Pitt, 1934], except that the slit then introduced to limit the width of spectrum entering the eye was dispensed with and the normal exit pupil used in its place. This was necessary in order to secure the high intensity test field required if the apparent intensity after light adaptation were not to be too low. The use of this exit pupil had the disadvantage that a rather wide band of wave-lengths, of the order of 0·0050μ, was admitted to the eye, but it is shown in the appendix given later that this introduces no appreciable error.

The observer viewed a 2° square test field divided horizontally into two equal parts, the upper half illuminated by a wave-length λ and the

lower half by, say, wave-length $(\lambda+\delta\lambda)$. To determine the size of the hue discrimination step at any point λ in the spectrum, the value of $\delta\lambda$ was increased until a just noticeable colour difference could be distinguished, at the same time ensuring that any intensity difference was removed by appropriate adjustment of the intensity of the top half of the field. When the reading $(\lambda+\delta\lambda_1)$ on the wave-length scale had been taken, the experiment was repeated in the opposite direction to give a second value $(\lambda-\delta\lambda_2)$, at which a colour difference could again just be seen. In general, $\delta\lambda_1$ and $\delta\lambda_2$ are very nearly equal and a mean value of $\delta\lambda$, given by

$$\delta\lambda=\tfrac{1}{2}\left[(\lambda+\delta\lambda_1)-(\lambda-\delta\lambda_2)\right],$$

is taken as the size of the hue discrimination step at the wave-length λ.

To determine the effect of light adaptation, the adapting field of 5° subtense was introduced in place of the test field, as in the intensity discrimination experiments [Wright, 1935], and the eye adapted to a radiation of the desired colour and intensity. After a period of 3 min., the adapting field was instantaneously removed to reveal the bipartite test field. This was examined momentarily to detect a colour difference as described above. In order to maintain the state of adaptation of the eye, the test field could only be viewed for very short intervals of the order of a second, but in between these brief observations the light adaptation was continued and the apparatus adjusted to bring the setting for a just noticeable colour difference more nearly correct for the next observation.

After a few attempts the setting for a just noticeable difference could be found, provided the intensity of the fields was controlled as before to prevent an intensity difference being mistaken for a hue step.

Values of $\delta\lambda$ in both directions were usually taken, and the whole experiment was repeated. In one or two series of observations it was found more convenient to set the two fields at the same wave-length λ and then determine the size of the step in one direction only. This was done when the size of step was large.

In describing the results, the retinal intensities of both test colour and adaptation have been given in photons, allowance having been made for the size of the exit pupil in the apparatus.

Experiments are described in which red and green adaptations were employed. These were obtained from a Pointolite lamp and Ilford spectral filters. The white adapting beam had a colour approximating to that of a radiation with a colour temperature of 2800° K.

All the observations were made by the author.

Selection of observations

In contrast to the method adopted by Hamilton & Laurens [1923], it seemed to the author of less interest to determine the complete hue discrimination curve for a given state of adaptation than to investigate the effect of various intensities of adaptation at selected points in the spectrum. Both sets of data are doubtless of value, but the former involves too many factors for the results to yield information of immediate theoretical significance. As it is, the possible number of combinations of adaptation and test colour are enormous, and if the problems involved are not to be overwhelmed by a mass of results, it is very necessary to restrict the experiments and variables to a few representative observations.

There are three possible ways in which adaptation might be expected to affect the value of $\delta\lambda$. First, particularly in the case of coloured adaptations, the relative proportions of the three colour responses, the red, green and blue, may be altered; thus a red adaptation will reduce the magnitude of the red response, making a yellow test colour look green, in which case a larger value of $\delta\lambda$ will be required to produce an equivalent change in the relative magnitudes of the red and green responses. Secondly, at high intensities of adaptation, the apparent intensity of the test colour will approach the threshold value, causing an increase in $\delta\lambda$. For example, in the limiting case when the test colour is only just visible, no colour change could be detected however great the wave-length shift. Thirdly, adaptation may modify the colour sensitivity of the perception mechanism itself.

The chief theoretical interest lies in the last alternative. We know the other effects must occur, and no great interest attaches to knowing their exact magnitude. The observations were therefore selected with this in mind.

Representative parts of the spectrum from which to select the test colours are obviously red, yellow, green, blue-green and blue. Of these, the red and blue must be discarded, the red because the discrimination is very poor and the blue because of its low intensity. To some extent this also applies to the blue-green.

The most instructive adaptation to use is a white, since this produces a minimum change in the red-green-blue response ratios. Red and green adaptations have also been used to obtain comparative results, but are of less direct significance.

Results

The results of eight combinations of test colour and adaptation are shown in Figs. 1–8. In these diagrams the just noticeable wave-length difference $\delta\lambda$ is plotted against the adaptation intensity to which the eye was subjected. The value obtained before light adaptating is indicated by the arrow on the left of each diagram. All the results have been confirmed, but not always under identical conditions to enable the repeat data to be recorded in the same diagram. But in two diagrams two sets of observations are shown, corresponding to data obtained at different times, while Figs. 1 and 2 represent repeat experiments for two different intensities of the same yellow test colour.

Discussion

Figs. 1–4 give the results for a white adaptation and are therefore of most importance. Figs. 1 and 2 show that the adaptation has increased the sensitivity of the eye so that a wave-length difference of only $0{\cdot}0004\mu$ (at $\lambda = 0{\cdot}58\mu$) can be detected as a colour difference. At high adaptation intensities, $\delta\lambda$ increases as the apparent brightness of the test colour decreases, and this effect is more marked, as might be expected, with the less intense test colour (Fig. 2). The combined effect of these two processes is to produce a curve which falls to a minimum value of $\delta\lambda$ and then rises again.

The same general effect is shown when a green test colour is used, as in Fig. 3. The curve shows a definite minimum, but the initial effect of the adaptation is different, in that the adaptation value of $\delta\lambda$ is greater than the pre-adaptation observation. The first effect of the adaptation was to decrease the sensitivity, but this action was reversed as the adaptation intensity increased, until the reduced apparent intensity of the test colour caused the curve to rise again.

With the blue-green test colour, lower intensities of both test colour and adaptation had to be used, and the main result of adapting the eye was to produce a grádual increase in $\delta\lambda$ (Fig. 4). There is no evidence of a minimum.

Figs. 5 and 6 show the very different action of green and red adaptations when using a yellow test colour. Both radiations produce a considerable increase in $\delta\lambda$, but while with the green the increase is continuous, with the red the curve falls to a minimum before rising again. It would be expected that the more intense the coloured adaptation the greater would be the specific depression of one or other of the responses and thus

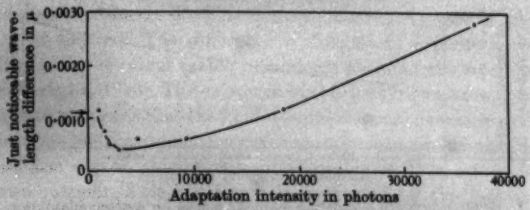

Fig. 1. Relation between adaptation intensity and $\delta\lambda$ for a white adaptation and a test colour of wave-length 0·58 μ and intensity 900 photons.

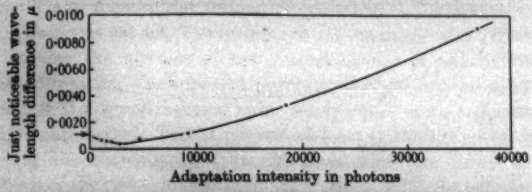

Fig. 2. Relation between adaptation intensity and $\delta\lambda$ for a white adaptation and a test colour of wave-length 0·58 μ and intensity 500 photons.

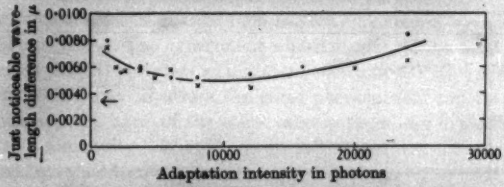

Fig. 3. Relation between adaptation intensity and $\delta\lambda$ for a white adaptation and a test colour of wave-length 0·53 μ and intensity 550 photons.

(The dots and crosses represent observations taken on different days.)

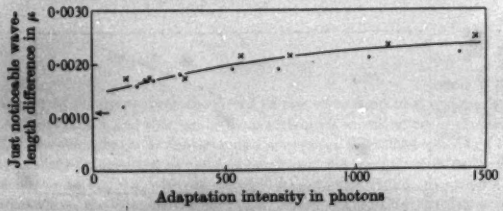

Fig. 4. Relation between adaptation intensity and $\delta\lambda$ for a white adaptation and a test colour of wave-length 0·494 μ and intensity 75 photons.

(The dots and crosses represent observations taken on different days.)

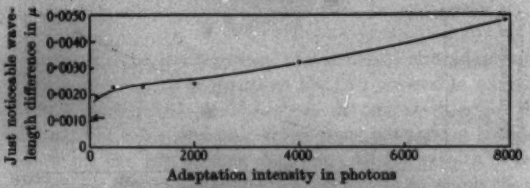

Fig. 5. Relation between adaptation intensity and $\delta\lambda$ for a green adaptation and a test colour of wave-length 0·58 μ and intensity 500 photons.

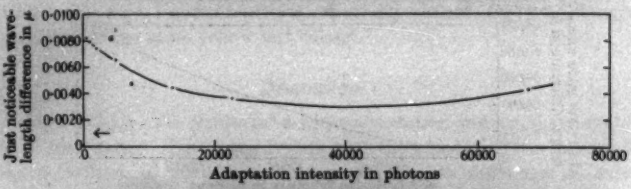

Fig. 6. Relation between adaptation intensity and $\delta\lambda$ for a red adaptation and a test colour of wave-length 0·58 μ and intensity 1080 photons.

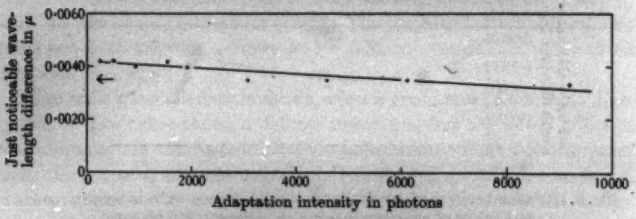

Fig. 7. Relation between adaptation intensity and $\delta\lambda$ for a green adaptation and a test colour of wave-length 0·53 μ and intensity 550 photons.

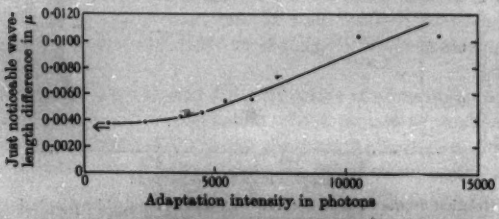

Fig. 8. Relation between adaptation intensity and $\delta\lambda$ for a red adaptation and a test colour of wave-length 0·53 μ and intensity 550 photons.

the greater the value of $\delta\lambda$. This is exemplified in the case of the green but not of the red. The difference is difficult to explain, unless it is linked up with the colour match breakdown phenomenon described elsewhere [Wright, 1936]. The existence of this breakdown at high adaptation intensities means that the fundamental response curves are modified, and since the hue discrimination curve must be intimately linked up with the response curves, this implies an alteration of $\delta\lambda$ in an, at present, unknown manner. This conclusion is supported by the fact that the breakdown principally affects the red response, and the unexpected decrease in $\delta\lambda$ is associated with the red adaptation.

Figs. 7 and 8 show the actions of green and red adaptations when using a green test colour. The results are in accordance with expectations if we assume the absence of any general change in sensitivity. With a green adaptation, the apparent proportions of red and green in a test colour of 0·53 μ would become more nearly equal and a reduction in $\delta\lambda$ would be anticipated. This is present at least to the extent of counterbalancing the increase that should arise from the lower apparent brightness of the test colour at high adaptations. With the red adaptation the reverse action showing a steady increase in $\delta\lambda$ was found, although no material increase occurred at the initial adaptation.

The evidence for a change of sensitivity produced by adaptation *per se* is conflicting. The experiment whose results can be most directly interpreted (Fig. 1) indicates an increased sensitivity. Figs. 3 and 5 show an effect which may illustrate the same phenomenon, but its origin is more obscure. In none of the other cases is there any evidence for an increase in sensitivity, although, as already stated, the interpretation is confused by other factors.

Even in Fig. 1, although the step diminishes to 0·0004 μ, this only represents an increase in sensitivity by a factor of $2\frac{1}{2}$, while the adaptation level is varied by as much as 40 : 1.

Appendix

It has still to be shown that the width of the wave-band used could not materially affect the observations, and this can be demonstrated if we consider, for example, the case of Fig. 6. Here the value of $\delta\lambda$ for the initial adaptation intensities was found to be about 0·0080 μ; the light transmitted by the exit pupil from the two halves of the field will therefore be as shown in Fig. 9 *a*, whereas the ideal transmission would be as shown in Fig. 9 *b*. Before adaptation, the integrated colour of *a* (1) and *a* (2) can be regarded as the same as that of *b* (1) and *b* (2) respectively. Is the same true after adaptation? In the case of Fig. 6, in which a red adaptation had been used, a change in the luminosity curve is produced, with

a preponderating reduction at the red end of the spectrum. It is therefore certain that if, before adaptation, a (1) had the same colour as b (1), then after adaptation they will have different colours. The integrated colour of a (1) will be displaced to the green side of 0·58 μ. It could never be displaced beyond 0·5775 μ, the extreme wave-length admitted, and the displacement could, in fact, hardly exceed half that amount. Suppose that after red adaptation, a (1) was equivalent to a strictly monochromatic beam of wave-length 0·5790 μ. The mean wave-length of a (2) is also displaced in a similar direction, though not necessarily to exactly the same extent. But the difference of displacement, which is the error in $\delta\lambda$, is not likely to be more than 0·0001 or 0·0002 and in an extreme case could hardly amount to more than 0·0005 μ. This is a second order error that can safely be neglected in relation to the value of $\delta\lambda$ of 0·0080 μ.

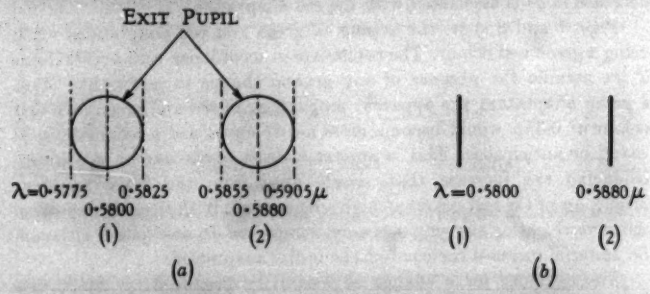

Fig. 9. Diagram to illustrate the wave band transmitted (a) by the exit pupil as used, and (b) in the ideal arrangement. (1) and (2) refer to the two beams from the two halves of the bipartite field when adjusted for a just noticeable difference after red adaptation.

It is less evident that the same conclusion will apply even to the case of Fig. 1, where a step of only 0·0004 μ was obtained. The same conclusions do apply, however, for the reason that the error is small, not only in relation to the width of spectrum admitted by the exit pupil, but also relative to the value of $\delta\lambda$ observed. The initial value of $\delta\lambda$ of 0·0010 μ could not be affected to an extent greater than 0·0001 μ even with a saturated green or red adaptation. Actually, in this experiment, a white adaptation was used, and as this will produce only a small change in the luminosity curve, the error in $\delta\lambda$ will be very small indeed.

It is therefore safe to conclude that in none of the experiments carried out could the magnitude of the wave band introduce any material error in the value of $\delta\lambda$.

Summary

Experiments are described in this paper in which the eye is adapted to a fairly high intensity and is then allowed to view momentarily a bipartite field of somewhat lower brightness. The wave-length change, $\delta\lambda$, required to produce a just noticeable difference of hue is determined with the eye adapted to various intensities and colours and for test

colours selected at three different points in the spectrum. In this way the adaptation is controlled in a known manner and its effect on the hue discrimination function determined. Analysis of the results shows three separate actions taking place: (*a*) a change in $\delta\lambda$ due to the change in shape of the response curves after adaptation to a coloured stimulus, (*b*) an increase in $\delta\lambda$ at high adaptation intensities due to the great reduction in apparent brightness of the test colour, and (*c*) a tendency in certain experiments for adaptation *per se* to increase the sensitivity of the visual mechanism and thus reduce the value of $\delta\lambda$.

The author desires to express his appreciation of the assistance given by Mr J. H. Nelson and to thank the Medical Research Council for their financial assistance.

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HUE DISCRIMINATION IN NORMAL AND ABNORMAL COLOUR VISION

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(*Received June* 14, 1936)

Discrimination of colour in the spectrum has been studied by many workers, amongst whom are Jones [1917], König & Dieterici [1884], Laurens & Hamilton [1923], Mandelstamm [1867], Pitt [1935], Roaf [1927], Steindler [1906], Uhthoff [1888] and Wright & Pitt [1934].

The results obtained show a certain extent of agreement between the various workers, but there are noticeable differences in the interpretation of the curves of discrimination. The classical experiments of Steindler [1906] gave a curve with four maxima of discrimination, whereas Roaf [1927] showed a curve with only two maxima. The existence or absence of the two extra maxima is part of the subject of discussion in this paper.

In the experimental investigation of sensory phenomena two courses may be adopted. Either one can make detailed observations on a few trained observers or one can make use of a larger number of less experienced individuals. In our opinion the more accurate results are obtained by the former procedure, but the latter shows whether or not the results obtained by the former method may have a more general application; hence both kinds of observations have been made during the present research.

Apparatus and technique

The apparatus used was similar to that employed by Laurens & Hamilton [1923]. It consisted of the collimators and prisms from two Hilger constant deviation spectrometers, both the telescopes being removed. On an extension to one of these spectroscopes was mounted a telescope consisting of lens and a small viewing hole (0·38 mm. in diam.). The viewing hole was at the focal plane of the lens; thus one obtained what is known as the Maxwellian view. A Lummer-Brodhun cube was

placed between the prism and the lens of the telescope, so that one saw one part of the design of the cube illuminated with light that had passed through the prism on that stand. The second stand was placed at right angles to the first and at such a position that the path of light from the

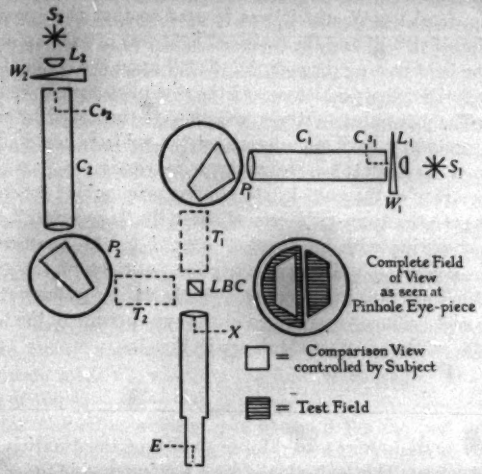

Fig. 1. Plan of apparatus used for the determination of hue sensitivity curves. Two Hilger constant deviation spectrometers were used with their telescopes removed. S_1 and S_2 are the two sources of light. L_1 and L_2 are cylindrical lenses of short focal length. W_1 and W_2 are wedges for the control of the brightness of the fields. C_1 and C_2 are the two collimators with their slits Cs_1 and Cs_2. P_1 and P_2 are the two constant deviation prisms. T_1 and T_2 are the arms from which the telescopes had been removed and replaced by covered spaces to prevent access of stray light. *LBC* is the Lummer-Brodhun cube. *X* is the lens of the telescope. *E* is the exit pupil 0·38 mm. in diameter. The width of the slit Cs_1 was adjusted so that the range of wave-lengths visible was about 24 Å. at 5853 Å. Slit Cs_2 at the same wave-length allowed a band of about 40 Å. to be visible. Inset: Field of view at the exit pupil showing the two patterns; one due to the fixed wave-length of the test field, and the other due to the variable wave-length controlled by the subject.

second prism to the side of the cube was equal to that from the first prism to the cube. The other portion of the design was thus illuminated by light from the second prism. When the two lights were of identical wave-length and intensity the prism was evenly illuminated and no trace of the design was visible.

The collimator slits were illuminated by 100-watt gas-filled 230-volt lamps, each mounted in a horizontal position so that a straight portion of the filament could be made parallel to the slit of its corresponding collimator. In order to secure adequate illumination of the cube a cylindrical lens was mounted between the lamp and the slit. The lens was about $\frac{3}{4}$ in. focal length, and it was focused so that there was intense illumination of the slit and the wedge of light was of such an angle that most of the light passing through the slit fell upon the collimator lens.

Between the cylindrical lenses and the slits photometric wedges were mounted. The movements of these wedges were controlled by racks and pinions operated by flexible leads so that the subjects could adjust the intensities of the lights whenever that was necessary.

The accuracy of the wave-length reading was verified by comparing the readings on the drum with certain lines in the "copper arc". This was done by observing with an eyepiece coaxial to the aperture in the telescope. Before each sitting the relative positions of the two collimators were controlled by observing that the two neon lines at 5853 Å. (one from each collimator) coincided at the eye aperture of the telescope.

The plan of the apparatus is shown in Fig. 1.

Methods

The observations were made at a single sitting, the duration of which was about 1 hour. This limitation of time was imposed in order to avoid inaccuracies due to fatigue. The subject was instructed to equalize the brightness of the two portions of the field at each step in the spectrum before any colour discrimination was made. This adjustment was made by means of the flexible controls to the photometric wedges. The only exceptions to this rule were when the two fields were made definitely of unequal intensities for a special purpose to be discussed later.

The observations were made traversing the spectrum either from the long to the short wave-length end or in the reverse direction for each of the single determinations carried out on the twenty-six untrained subjects. For the few trained subjects the spectrum was traversed on a number of occasions and in both directions.

The experimenter presented to the subject a light of a fixed wave-length from one collimator. The subject was asked to alter the wave-length of the other portion of the field until there was a barely perceptible difference in hue: that is, after equalizing the brightnesses as described above.

It was soon found that the manipulation of the wave-length drum of the prism P_1 was too coarse for accurate determinations, therefore an endless band was passed round the edge of the drum and also round a groove on the face of a larger wheel. The larger wheel was at a convenient distance from the subject, and by turning it the movement was transmitted to the wave-length drum at a 4 to 1 ratio, hence coarse movements of the larger wheel gave fine movements of the wave-length drum, thus making possible more accurate measurements.

After a first reading of hue difference was made the subject was instructed to turn the controlling wheel until a noticeable difference in hue was observed on the opposite side of the fixed wave-length, any difference in brightness being eliminated during the observation. This procedure was repeated three times, giving six readings, three on the long and three on the short wave-length side of the fixed wave-length. These readings were usually concordant, and probably the best value to use was the medium of each group of three. The readings were much closer together with normal subjects at those regions where the discrimination was fine. Where the discrimination was coarse and with hypochromats there was more "scatter" amongst the values of the readings. The readings were made after setting the fixed wave-lengths at successive intervals of 100 Å.

By this procedure a value was obtained for the just perceptible difference in hue on opposite sides of the fixed wave-length. The difference between these values divided by 2 gave the difference in wave-length required for discrimination at that fixed wave-length. Let the fixed wave-length be represented by λ and the two values on either side of it be λ_1 and λ_2 respectively, then the value for the step of discrimination at λ will be $(\lambda_2-\lambda_1)/2$. Further, if this value be added to λ_1 we obtain the value $\lambda_1+\frac{\lambda_2-\lambda_1}{2}=\frac{\lambda_1+\lambda_2}{2}$; therefore if the two values are added and divided by 2, a figure is obtained which should be approximately equal to λ. The significance of this approximation will be discussed later with reference to observations in which the intensities of the two fields were made unequal on purpose.

Previous workers have used other methods for evaluating the size of the step of discrimination. Steindler [1906] traversed the spectrum only in the direction from the long to the short wave-length end. Her measurement was merely the difference in wave-length between the two observations. If she had traversed the spectrum in the opposite direction different values would have been obtained according to whether the

readings were approaching to or receding from a maximum of discrimination.

König & Dieterici [1884] made the two halves of the field appear to be of the same colour. This method has been discussed by Martin *et al.* [1933], and they show a mechanical analogy to illustrate the difference between making the observations to similarity or dissimilarity of hue. Laurens & Hamilton employed the method of "right or wrong answers". This will give a lower threshold, but is more apt to give values depending upon differences in intensity as the subject is concentrating on observing whether or not there is a difference rather than on whether it is or is not a difference in hue.

Wright & Pitt [1934] discussed these points and they finally adopted the method used in the present research. We press the point that the method of using the average of the differences on both sides of the fixed wave-length is more likely to be correct than the value obtained on one side of the fixed wave-length only. The methods of "similarity" and of "right or wrong answers" may give lower threshold values, but they do not give the true values for recognizable difference in hue.

Hue discrimination in normal colour vision

Trained normal subjects. *Results*

These were subjects who made observations on a number of occasions, and it is presumed that greater accuracy is obtained by practice.

The observations on H. E. R. were repeated on fourteen different occasions with the intensities equalized at and during each set of readings. Curves 1–4 show examples of the results.

Curve 1 (28. v. 34) (Fig. 2) shows the result of traversing the spectrum from the long to the short wave-length end. There are two maxima of discrimination about 5000 and 6000 Å. respectively. There is a doubtful third maximum at 4700 Å. Two months later a similar result was obtained (curve 2, Fig. 2), but the third maximum was at 4600 Å. instead of 4700 Å.

Curves 3 and 4 (Fig. 3) show the result of traversing the spectrum in the reverse direction: neither of these curves shows any evidence of subsidiary maxima below 4900 Å. or above 6000 Å. respectively. This subject has had more experience and practice at this work than any of the others studied here, so this absence of subsidiary maxima may be of some significance.

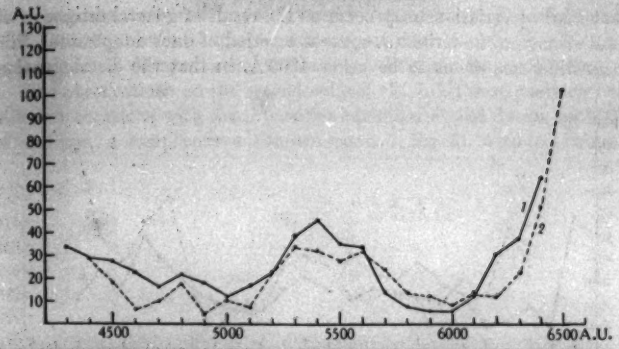

Fig. 2. Curve 1. Hue discrimination of H. E. R. on 28. v. 34 (see curve 15). Curve 2. Hue discrimination of H. E. R. on 27. vii. 34. Both scaled in the red to violet direction with equalized brightnesses.

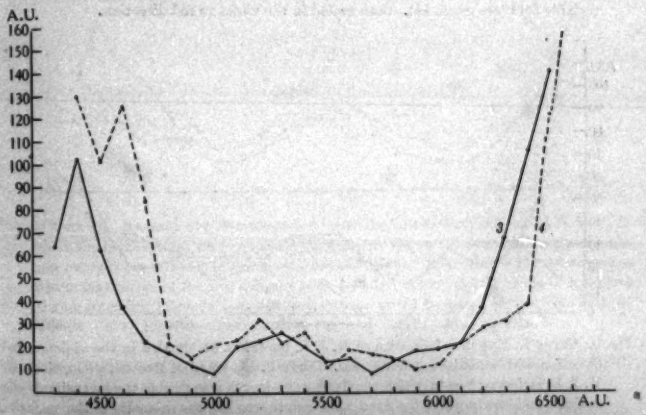

Fig. 3. Curve 3. Hue discrimination of H. E. R. on 18. vi. 34 (see curve 16). Curve 4. Hue discrimination of H. E. R. on 13. vii. 34. Both scaled in the violet to red direction with equalized brightnesses.

Curves 1–4 (Figs. 2 and 3) show the variations obtained with the same individual at different times and under different conditions. Judging by the results of spatial discrimination by touch it is probable

that marked variations may occur as the result of general fatigue or of local effects on the retina (in spite of a period of dark adaptation). The main difference seems to be below 4600 Å. in that the discrimination

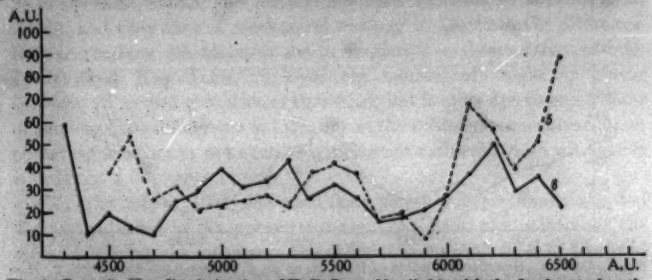

Fig. 4. Curve 5. Hue discrimination of H. E. R. on 11. vii. 34, with the fixed wave-length half the brightness of the variable field (see curve 13). Curve 6. Hue discrimination of H. E. R. on 12. vii. 34, with the fixed wave-length double the brightness of the variable field (see curve 14). Both scaled in the violet to red direction.

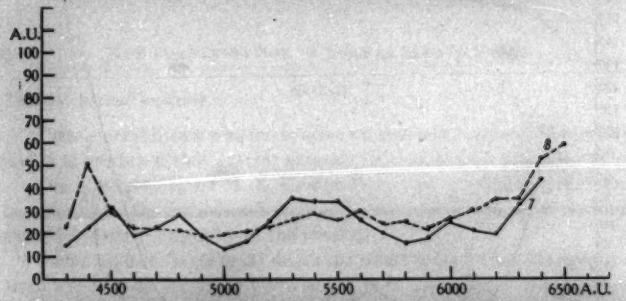

Fig. 5. Curve 7. Hue discrimination of R. J. L. on 28. vi. 34. Scaled in the violet to red direction with equalized brightnesses. Curve 8. Average of five determinations by R. J. L. including two in which the fixed wave-length was double the intensity of the variable and two in which the fixed wave-length was half the intensity of the variable.

seems more acute when the spectrum is traversed from the long to the short wave-length end. It is difficult to know whether this is the result of fatigue or of "successive contrast".

Curve 9 (Fig. 6) shows the average of the curves 1–4.

H. E. R. noted that hue discrimination was very difficult at the extremes of the spectrum, as it was difficult to decide whether a given

difference was really due to a difference in hue or to unequal brightness. This difficulty obtains experimental support when the results of observations on hue discrimination with unequal brightnesses are discussed.

The observations on the second subject (R. J. L.) were made on five different occasions with the intensities equalized at and during each set of readings. A sample curve is shown (curve 7, Fig. 5). With this subject

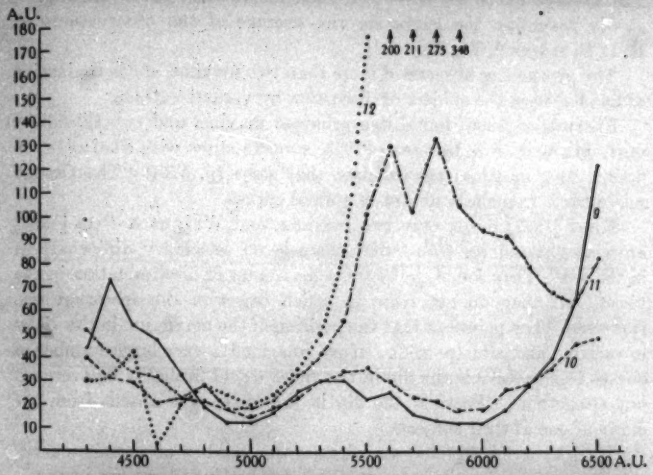

Fig. 6. Curve 9. Average hue discrimination curve for four determinations by H. E. R. in which the brightnesses were equalized throughout. Curve 10. Average hue discrimination curve for seventeen subjects with normal colour vision. Curve 11. Average hue discrimination curve for four subjects with defective colour vision. If the fifth subject (shown by curve 12) were included this average would be raised higher from 5300 to 6500 Å. Curve 12. Hue discrimination curve of H. K. L. on 3. vii. 34.

there is evidence of four maxima when traversing the spectrum both from the long to the short and from the short to the long direction.

An average curve for five sets of observations on R. J. L. is shown in curve 8 (Fig. 5).

Untrained normal observers.

These subjects may not have had previous experience in colour discrimination, but they were all workers in medicine or science, which means that they had had experience in the use of microscopes or other

optical apparatus. Subjects who are not practised in looking through "tubes" would be greatly handicapped in such observations as those presented here.

In spite of individual variations in the general form of the curve there are always two maxima of discrimination, a broad one between 5700 and 6000 Å. and a somewhat sharper one between 4800 and 5000 Å. The average curve for seventeen such individuals (curve 10, Fig. 6) closely resembles the curve for the average of the observations on H. E. R. (curve 9, Fig. 6).

The presence or absence of more than two maxima of discrimination of hue has been the subject of discussion by various authors.

Steindler [1906] found two principal maxima and two subsidiary ones. Hamilton & Laurens [1923] seem to agree with Steindler in finding four maxima; nevertheless, they state (p. 576): "The Orange minimum is frequently absent in normal curves."

Roaf [1927] found only two maxima, and Wright & Pitt [1934] have also stated (p. 463) "that there is no secondary dip whatever in the red". They found, however, a maximum of discrimination in the violet, but they do not state in which direction the spectrum was traversed. They point out that the position of the maximum in the violet is variable and add (p. 464), "if we imagined a very large number of curves to be available the dip in the violet would probably be averaged out altogether". Further, the dip in the violet was absent from the curve of one of their subjects.

Wright & Pitt [1934] also discuss the validity of average curves obtained from a number of separate individuals, and we are in agreement with what they say (p. 464): "Hence an average curve may not necessarily represent the visual characteristics of a normal eye."

Unfortunately it is not possible to publish all the curves from such a large number of individuals, hence the average is given in curve 10 (Fig. 6).

Variations in the curves are due to so many factors that the average is useful in showing the general trend of all such observations. In previous work by Roaf [1927, 1932] a new method of investigating the validity of colour discrimination was described. This consisted in making one spectrum distinctly different in brightness from the other. Colour discrimination curves obtained in this way show evidence of four maxima even when the spectrum is traversed in the short to the long wavelength direction. This result suggests that the secondary maxima are somehow related to differences in brightness in spite of attempts to equalize the brightness when making hue discrimination measurements.

Very striking results are shown (curves 13, 14, 15 and 16, Fig. 7) when the shift in the value $\frac{\lambda_1+\lambda_2}{2}$ (see p. 179) is plotted against λ. When the intensities are equalized the "shift" is not very great at any wave-length but becomes noticeable at the extremes of the visible spectrum. When the intensities are unequal the "shift" is well marked except close to the

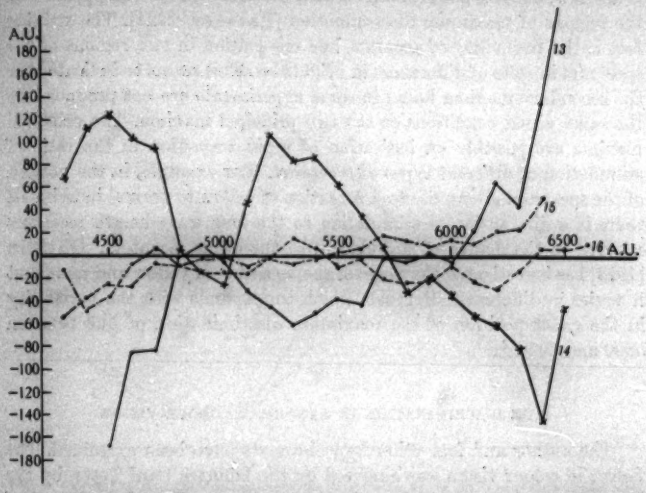

Fig. 7. "Shift" for an individual with normal colour vision. Curve 13. "Shift" in matching of wave-lengths when the fixed wave-length was half the intensity of the variable one (H. E. R. 11. vii. 34. See curve 5). Curve 14. "Shift" in matching of wave-lengths when the fixed wave-length was double the intensity of the variable one (H. E. R. 12. vii. 34. See curve 6). Curve 15. "Shift" in matching of wave-lengths when the brightnesses were equalized throughout (H. E. R. 28. v. 34. See curve 1). Curve 16. "Shift" in matching of wave-lengths when the brightnesses were equalized throughout (H. E. R. 18. vi. 34. See curve 3).

region where the two main maxima of discrimination are found. This result furnishes very clear evidence that at these regions of the spectrum (4800–5000 Å. and 5700–6000 Å.) hue discrimination is definite and acute in spite of differences in brightness, whereas at other regions intensity differences have a marked effect on hue discrimination. The "shift" is always away from the brightest part of the spectrum, so that

the dimmer spectrum is always matched with a point on the brighter spectrum farther away from its brightest point. As shown on curves 13 and 14 one is the mirror image of the other because in curve 14 the fixed wave-length was brighter than the variable one and in curve 13 it was dimmer. The statement that reduced brightness always causes "shift" in the hue discrimination away from the brightest part of the spectrum is not new, but it is not generally stated that the "shift" disappears near the regions of maximum discrimination [Parsons, 1924]. The striking fact is the possibility of accurate hue recognition in two regions of the spectrum in spite of differences in brightness. This seems to indicate that the secondary maxima found in some experiments are not produced by the same visual conditions as the two principal maxima. The principal maxima are possibly an indication of rapid transition in the ratio of stimulation of different types of receptors. For example, in the portion of the spectrum giving rise to a sensation of yellow to normal individuals there is a diminution of stimulation to the long wave-length receptors relative to the degree of stimulation to the other receptors. Göthlin [1923] has investigated the range of the sensation of yellow and finds that it varies in different individuals which corresponds with the variability in the exact position of the maximum discrimination of hue between 5800 and 6000 Å.

Hue discrimination in abnormal colour vision

The author and four other hypochromats have been examined. The defect in colour vision was analysed by the Ishihara Card Tests, by the Nagel Anomaloscope and by a lantern similar to that of the Board of Trade lantern.

All five subjects were cases of red-green confusion. H. V. C. requires an excessive proportion of red in the red-green mixture which matches the fixed yellow of the anomaloscope, whereas the other four require an excess of green in the mixture. These differences can be explained by a decreased brightness of the red end of the spectrum in the former case and an increase in the four latter ones, and this supposition is supported by the fact that when the match is made the brightness of the yellow must be diminished in the former and increased in the latter. These groups are sometimes called protanope and deuteranope respectively, but it is preferable to use terms which are not construed as red and green blind respectively. Rivers' terms scoterythrous and photerythrous are more descriptive and do not imply any theoretical significance.

Pitt [1935] agrees with this view in that the luminosity curves of protanopes show relatively less brightness at the long wave-length end of the spectrum.

The results obtained (curves 17, 18, Fig. 8) show that the hypochromat has a maximum of discrimination about 4900 Å., thus agreeing with that for normal colour vision. At the long wave-length end there

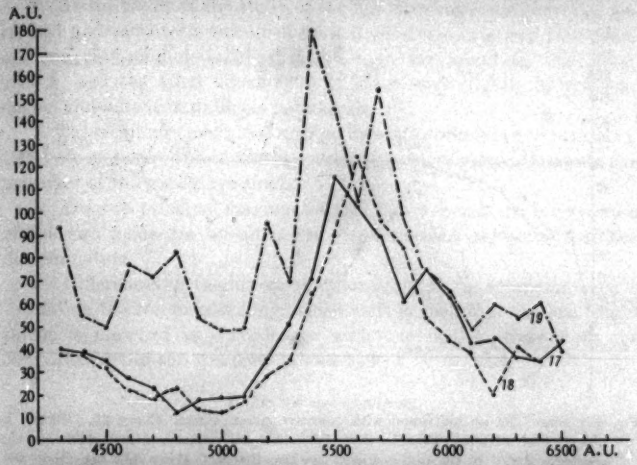

Fig. 8. Curve 17. Hue discrimination of H. V. C. on 21. vi. 34 when the brightnesses were equalized throughout and the spectrum scaled in the violet to red direction (see curve 21). Curve 18. Hue discrimination of H. V. C. on 31. v. 34 when the brightnesses were equalized throughout and the spectrum scaled in the red to violet direction (see curve 20). Curve 19. Hue discrimination of H. V. C. on 7. vii. 34 when the intensity of the fixed wave-length was half that of the variable one and the spectrum scaled in the red to violet direction (see curve 22).

is no maximum about 5900 Å., although the curve shows a fall at the extreme of the spectrum. This fall does not indicate a true maximum of discrimination but is probably a brightness effect. Clearly at the ends of the spectrum the possibility of error in one direction is limited by the decrease in brightness of the spectrum, therefore one might expect a downward slope at the red end of the curve.

Curves 17 and 18 show the discrimination curve of H. V. C. when the brightnesses of the two fields are matched throughout the observations.

Curve 19 shows the hue discrimination curve of H. V. C. when the intensity of the fixed wave-length was half that of the variable one. His discrimination was disorganized by the difference in the intensity.

The great importance of the intensity of the light is shown in curves 20, 21 and 22 (Fig. 9). When the intensities are balanced the wave-lengths of the fixed standard and the average between the minimal

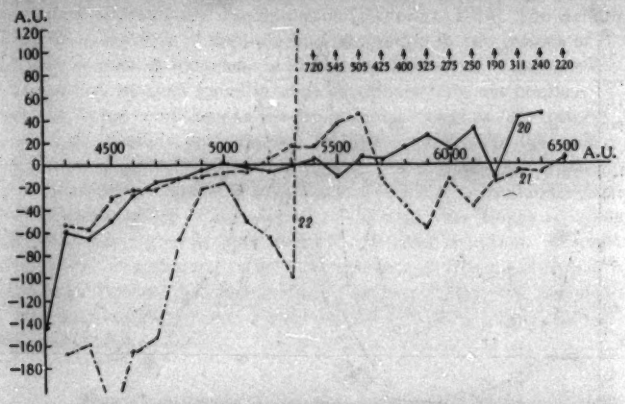

Fig. 9. "Shift" for an individual with defective colour vision. Curve 20. "Shift" in matching of wave-lengths when the brightnesses were equalized throughout and the spectrum scaled in the red to violet direction (H. V. C. 31. v. 34. See curve 18). Curve 21. "Shift" in matching of wave-lengths when the brightnesses were equalized throughout and the spectrum scaled in the violet to red direction (H. V. C. 21. vi. 34. See curve 17). Curve 22. "Shift" in matching of wave-lengths when the intensity of the fixed wave-length was half that of the variable one and the spectrum scaled in the red to violet direction (H. V. C. 7. vii. 34. See curve 19).

differences, $\frac{\lambda_1+\lambda_2}{2}$, correspond, but when the intensities are not balanced a marked shift occurred except near the maximum of discrimination about 4900 Å. Once the colours are in the longer wave-lengths all hope of a correct match disappears with the result shown in curve 22. It is to be noted that with one of the hypochromats all hope of colour discrimination disappears with the longer wave-lengths (curve 12, Fig. 6), although the brightnesses were supposed to be balanced by the subject.

The average hue discrimination curve for four hypochromats is shown in curve 11 (Fig. 6). If the curve for the fifth (shown in curve 12)

were included the average curve for the five would have coincided with that for the four from 4300 to 5300 Å. but would have been at a higher level from 5300 Å. to the limit of the observations on this fifth subject.

Summary

Normal colour vision

1. As the result of the study of the hue discrimination curves of ten trained and seventeen untrained normal observers it is seen that there are two real maxima of discrimination in the spectrum, one about 4900 Å. and the other about 6000 Å. These vary slightly in position, degree and extent in different individuals.

2. The secondary maxima described by some workers [e.g. Steindler, 1906] are possibly due to the rapid decrease in brightness towards the extremes of the visible spectrum.

3. The two principal maxima are still found even if the brightnesses of the two fields are unequal, and these maxima represent real hue discrimination.

4. Differences in brightness at other parts of the spectrum cause a "shift" in the colour matching, which shift is such that the less bright colour is matched in wave-length with one farther away from the brightest part of the spectrum (about 5900 Å.).

Defective colour vision

As the result of a study of the hue discrimination curves of one trained and five untrained observers it is seen that:

5. Defective colour vision is associated with only one maximum of discrimination which is situated at about 4900 Å.

6. Differences in intensity cause more difficulty in hue discrimination for hypochromats than for those with normal colour vision.

7. The "shift" in colour matching for hypochromats is very striking except at the one maximum of discrimination about 4900 Å. This result shows that there is no indication of a second maximum of discrimination about 5900 Å.

The author thanks Prof. Roaf for suggesting the subject of research and for his continued interest in its progress. He also thanks the staff and those students of the Department of Physiology who helped in the research.

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THE EFFECT OF ADRENALINE ON MUSCLE GLYCOGEN IN ADRENALECTOMIZED, THYROIDECTOMIZED, AND HYPOPHYSECTOMIZED RATS

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(*Received August* 4, 1936)

Braier [1931], Corkill *et al.* [1933] and others have noted that adrenaline may partially or wholly fail to cause elevation of the blood sugar in hypophysectomized animals. Since the increase in blood lactate is also subnormal [Chaikoff *et al.* 1935*a*; Marks, 1936], and since the muscle glycogen is the chief source of the sugar mobilized by adrenaline in normal animals, it appeared probable that in hypophysectomized animals the muscle glycogen is relatively resistant to the action of adrenaline; Bachman & Toby [1936] reported three experiments in which this action was studied in hypophysectomized rabbits under amytal anæsthesia. The object of this investigation was to study the phenomenon more closely, and especially to evaluate the parts played by atrophy of the thyroid and the adrenal cortex respectively in the alterations in carbohydrate metabolism characteristic of the hypophysectomized animal.

Material and methods

The experiments were carried out on hooded rats 2–3 months old, the great majority being males. As it was considered inadvisable to risk the effect of fasting on hypophysectomized and adrenalectomized animals, food ("Purina chow") was not withdrawn before the experiments; no attempt was made to secure constancy of intake, but the experiments were always started at the same time of day.

The rats were anæsthetized with sodium amytal, injected intraperitoneally: 80–100 mg. per kg. body weight in the intact animals, while 50–80 mg. usually sufficed for the operated rats. Muscle samples were obtained by the technique described by Cori [1930]; when relaxation was complete and tactile stimulation no longer evoked twitches, the rat

was tied down, the skin of the leg split, the tendo Achillis clamped with forceps and cut; the soleus was then separated, the gastrocnemius set free by a single cut across the tendons at the upper end and dropped immediately into 30 p.c. KOH for weighing. 0·25 mg. of adrenaline per kg. of body weight was then injected subcutaneously; 3 hours later the second gastrocnemius and a portion of liver were removed for analysis.

Glycogen determinations were carried out by the method of Good *et al.* [1933], the modified Shaffer-Hartman method described by Peters & van Slyke [1932] being used to determine the sugar formed, and also in the blood-sugar estimations.

The adrenalectomized rats were used before insufficiency symptoms developed; a number of them received normal saline in place of drinking water after operation. In the majority of the thyroidectomized rats, the metabolic rates were determined shortly before the experiment by the method employed by Anderson & Collip [1934] who obtained an average value of 166 l. oxygen per sq. m. body surface per 24 hours in normal rats of this colony. Hypophysectomy was carried out by the parapharyngeal route; a number of the animals available for these experiments had received treatment for various periods with anterior pituitary extracts of different kinds, but not for at least 3 weeks before coming into our hands. A special study was, however, devoted to hypophysectomized rats which had just received 5–10 days' treatment with an extract designed to exhibit thyreotropic activity [Anderson & Collip, 1933] in doses of 0·25–0·5 c.c. twice daily; two rats were similarly pretreated with an adrenotropic fraction [Collip *et al.* 1933] of undetermined potency, in which other pituitary hormones had been inactivated by boiling. Finally, a group of four hypophysectomized rats received 0·12 g. of desiccated thyroid orally for 5–7 days before use.

Results and discussion

In a series of nineteen normal rats, which were subjected to the amytal anæsthesia and the necessary manipulations but received no adrenaline, the gastrocnemius muscle glycogen decreased during the experiment by only $1·1 \pm 5·4$ p.c. of its initial value of 0·498 p.c., while the average of the final liver glycogen values was $2·8 \pm 1·97$ p.c. It is therefore evident that the injected adrenaline is largely responsible for the decreases in muscle glycogen reported in Table I, while it also caused a decrease in liver glycogen, presumably because of the use of amytal [Cori & Cori, 1929; Evans *et al.* 1931], the amytal in itself causing some reduction of liver but not of muscle glycogen. Studies on three adrenalectomized and three

TABLE I. Effect of adrenaline on normal rats

Muscle glycogen p.c.			Liver glycogen p.c.	Blood sugar mg./100 c.c.	
Initial	Final	Change	Final	Initial	Final
0·502	0·217	−57	1·28	—	—
0·574	0·289	−50	1·87	—	—
0·500	0·307	−39	0·60	—	—
0·500	0·298	−40	0·63	—	—
0·416	0·217	−48	1·50	107	215
0·483	0·229	−53	0·104	—	—
0·524	0·266	−49	0·172	—	—
0·730	0·345	−53	0·762	92	215
0·537	0·282	−48	0·088	96	264
0·535	0·264	−51	0·540	—	—
0·487	0·210	−57	1·54	96	211

hypophysectomized rats showed that the muscle glycogen underwent little change in the absence of injected adrenaline.

In the adrenalectomized rats, the proportion of the muscle glycogen mobilized by adrenaline was significantly greater than normal (69 against 49 p.c.); it is conceivable that in the normal animals, in which a large fraction of the liver glycogen was mobilized and there was persistent hyperglycæmia, some restitution of muscle glycogen took place during the experiment. It is more difficult to understand the fate of the muscle

TABLE II. Effect of adrenaline on adrenalectomized rats

Day after operation	Muscle glycogen p.c.			Liver glycogen p.c.	Blood sugar mg./100 c.c.	
	Initial	Final	Change	Final	Initial	Final
4	0·444	0·115	−74	0·026	107	92
4	0·360	0·125	−65	0·352	75	100
4	0·360	0·123	−66	0·029	92	141
4	0·445	0·121	−73	0·049	79	75
4	0·393	0·122	−69	0·029	83	75
4	0·440	0·126	−71	0·025	67	63
4	0·345	0·116	−67	0·025	75	67
5	0·434	0·125	−71	0·050	—	141
5	0·383	0·109	−71	0·112	92	174
5	0·478	0·167	−65	0·050	—	115
6	0·527	0·251	−52	0·062	—	100
6	0·443	0·115	−74	0·047	83	115
7	0·374	0·079	−79	0·024	88	—
7	0·244	0·070	−71	0·022	—	92
7	0·436	0·133	−70	0·118	92	149

glycogen mobilized, since only a small fraction can be accounted for as blood sugar or liver glycogen, especially in the adrenalectomized rats. Here it might be thought that the carbohydrate cycle was interrupted by inefficiency in the conversion of lactate into liver glycogen [Britton & Silvette, 1934; Buell *et al.* 1932, 1936; Asaeda & Shen, 1934], were

it not for other evidence that adrenaline produces less than the normal rise in blood lactate in adrenalectomized animals [Anderson, 1935; Thaddea, 1935]. It would be useless to attempt to construct a balance sheet for these experiments, lacking data on carbohydrate oxidation and excretion and with only a small sample of the whole mass of skeletal muscle available for study, yet it may be recalled that Corkill & Marks [1930] were unable to account for all the muscle glycogen mobilized by adrenaline in eviscerated spinal cats. In the absence of a series of values for liver glycogen in untreated adrenalectomized rats, it is not possible to say whether the apparent discrepancy is actually increased by adrenalectomy or not.

TABLE III. Effect of adrenaline on thyroidectomized rats

Day after operation	Muscle glycogen p.c.			Liver glycogen p.c.	Blood sugar mg./100 c.c.		Basal oxygen l./sq. m. body surface per 24 hours
	Initial	Final	Change	Final	Initial	Final	
7	0·389	0·250	−36	0·901	79	276	146
7	0·514	0·356	−31	0·030	—	280	166
10	0·353	0·337	− 4	1·12	104	119	128
10	0·578	0·468	−19	1·09	100	202	152
12	0·506	0·366	−27	1·07	104	338	—
12	0·411	0·455	+10	1·85	141	321	—
12	0·568	0·354	−38	0·448	124	231	—
13	0·357	0·317	−11	0·761	96	285	—
13	0·396	0·323	−18	0·309	104	235	—
13	0·387	0·340	−12	0·309	92	228	—
13	0·514	0·356	−31	0·030	—	280	166
15	0·500	0·410	−18	1·82	107	248	121
15	0·429	0·323	−25	0·108	119	256	152
19	0·564	0·500	−11	1·27	—	272	127
19	0·552	0·312	−43	1·03	115	370	141
28	0·437	0·241	−45	0·169	107	326	153
28	0·443	0·263	−40	0·840	111	285	139
31	0·399	0·304	−24	0·600	107	231	129
31	0·302	0·226	−25	0·764	107	276	129
31	0·421	0·348	−17	0·913	107	198	153
33	0·432	0·337	−22	1·14	100	313	132
33	0·455	0·368	−19	0·949	107	305	130
33	0·387	0·224	−42	0·562	115	297	150

The correlation between metabolic rate and p.c. change in muscle glycogen is just below the level of significance (0·417 ±0·211).

In the thyroidectomized rats, the hyperglycæmic response to adrenaline is at least equal to normal, if it is not greater [Inawashiro, 1933]. It seems probable that the initial levels of liver glycogen were at least as high as in the intact animals; and yet, since the peak of the hyperglycæmic curve in the latter may have been passed when the final blood sample was taken, the data presented do not necessarily conflict with the observation of Goldblatt [1936] that in thyroidectomized rabbits

treated with adrenaline the blood sugar rises more slowly and less high than usual. We are probably on firmer ground in postulating a sluggishness of the glycogenolytic mechanisms in muscle to account for the low value of the percentage decrease observed after adrenaline; for it is hardly plausible to suppose that normal mobilization has been masked by unusually rapid restitution in the absence of the thyroid.

A similar irresponsiveness of the muscle glycogen to adrenaline is seen to develop gradually after hypophysectomy; in which case the absence of

TABLE IV. Effect of adrenaline on hypophysectomized rats

Day after operation	Muscle glycogen p.c.			Liver glycogen p.c.	Blood sugar mg./100 c.c.		Adrenal weight mg.
	Initial	Final	Change	Final	Initial	Final	
21	0·305	0·136	−55	0·044	—	—	10
27	0·522	0·322	−38	0·108	—	—	14·5
27	0·345	0·251	−27	0·131	—	—	11·5
31	0·356	0·255	−28	0·060	—	—	11
31	0·201	0·132	−34	0·062	—	—	11
61	0·593	0·572	− 3	0·123	83	83	8
61	0·482	0·469	− 3	0·100	83	83	9
61	0·507	0·480	− 5	0·192	103	124	8
61	0·419	0·349	−17	0·125	92	119	9
61	0·307	0·265	−14	0·117	96	100	11
76*	0·425	0·401	− 6	0·312	—	—	10
76*	0·482	0·430	−11	0·672	—	—	8
76*	0·465	0·415	−11	0·677	—	—	7
122	0·453	0·377	−17	0·192	83	115	7
122*	0·466	0·373	−20	0·062	92	115	10
122*	0·369	0·381	+ 3	0·069	—	—	—
153*	0·435	0·354	−19	0·070	83	67	16
153*	0·308	0·219	−29	0·188	92	182	8
153*	0·471	0·410	−13	0·070	71	111	7
153*	0·419	0·333	−20	0·070	120	145	6
184*	0·253	0·188	−25	0·314	—	—	—
	Injected with adrenotropic hormone						
122*	0·462	0·426	− 8	0·098	115	182	9
122*	0·273	0·234	−14	0·069	115	198	9·5
	Injected with thyreotropic hormone						
77	0·488	0·271	−44	0·076	92	157	12
77	0·412	0·149	−64	0·058	100	224	11
77	0·427	0·217	−49	0·108	104	170	9
92	0·550	0·282	−48	0·331	115	272	11
92	0·557	0·246	−56	0·269	100	305	11
92	0·557	0·192	−66	—	83	190	10
92	0·500	0·294	−41	—	83	132	9
107*	0·444	0·252	−43	0·309	—	—	—
184*	0·540	0·314	−42	0·059	—	—	—
	Receiving desiccated thyroid orally						
70	0·364	0·190	−48	0·059	83	124	11
70	0·368	0·142	−61	0·053	115	154	8
70	0·395	0·114	−71	0·076	—	92	10
70	0·322	0·113	−65	0·147	87	83	11

* These animals had been previously treated with various pituitary extracts, but not within three weeks of the experiment reported.

hyperglycæmia makes rapid restitution still less likely. It is tempting to compare the hypophysectomized rats to animals from which both thyroid and adrenals have been removed: in the virtual absence of the hormone of the adrenal cortex there is neither enough preformed liver glycogen nor enough formation of liver glycogen from blood lactate or other sources to permit adrenaline hyperglycæmia; while the striking effect of feeding desiccated thyroid may be cited to prove that it is lack of thyroid hormone that protects the muscle glycogen against the injected adrenaline. The restoration of the hyperglycæmic response after treatment with anterior-lobe extracts, which was also observed by Cope & Marks [1934], might on this view be interpreted as an effect of the adrenotropic hormone, probably present as a contaminant of the thyreotropic extract; on the other hand, the adrenal weights do not indicate that much restoration of the cortex has taken place, and the final liver glycogen values remain surprisingly low. The two experiments with adrenotropic extract unfortunately contribute no definite information. One must not overlook the possibility that hypophysectomy has effects on carbohydrate metabolism independent of the secondary involution of the thyroid and adrenal cortex; thus Russell & Bennett [1936] claim that the rapid depletion of carbohydrate stores during fasting, so characteristic of the hypophysectomized animal, may be prevented by anterior-lobe extracts which are free from thyreotropic activity and active in the absence of the adrenals.

The values for muscle and especially liver glycogen naturally show more variation in these experiments than in studies on fasted animals. It may be shown nevertheless that the initial amounts of muscle glycogen are slightly but significantly less in the adrenalectomized, thyroidectomized and untreated hypophysectomized rats than in the controls, the

TABLE V. Averages and standard deviations

	Initial muscle glycogen p.c.	Decrease in muscle glycogen p.c.	Blood-sugar increase mg./100 c.c.	Final liver glycogen p.c.
Normal animals	(25) 0·510 ± 0·096	(11) 49·1 ± 6·2	(4) 128 ± 27	(11) 0·83 ± 0·63
Thyroidectomized	(23) 0·448 ± 0·076	(23) 23·8 ± 13·4	(20) 159 ± 57	(23) 0·79 ± 0·50
Adrenalectomized	(17) 0·409 ± 0·062	(15) 69·1 ± 6·2	(10) 20 ± 34	(15) 0·07 ± 0·08
Hypophysectomized:				
3 weeks	(1) 0·305	(1) 55	—	(1) 0·04
4 weeks	(4) 0·356 ± 0·131	(4) 31·7 ± 5·2	—	(4) 0·09
8 weeks	(16) 0·428 ± 0·084	(16) 12·6 ± 8·7	(11) 23 ± 28	(16) 0·20 ± 0·20
Treated with thyreotropic hormone	(9) 0·497 ± 0·058	(9) 50·4 ± 9·4	(7) 110 ± 57	(7) 0·17 ± 0·12
Treated with desiccated thyroid	(4) 0·362 ± 0·030	(4) 61·2 ± 9·7	(3) 25	(4) 0·08

The figures in brackets represent the number of measurements averaged.

differences between the various operated groups being negligible. Low glycogen values are characteristic of adrenalectomized animals [Britton & Silvette, 1932], and other data [Phillips & Robb, 1934; Chaikoff *et al.* 1935*b*] indicate a similar tendency in hypophysectomized animals, even without fasting. In the present experiments the administration of thyreotropic hormone significantly increased the muscle glycogen of the hypophysectomized rats to a value indistinguishable from the normal; since thyroid feeding quite failed to do so, this effect may be, in part at least, due to the adrenotropic or some other contaminating principle.

Summary

The injection of 0·25 mg. adrenaline per kg. body weight into normal non-fasted rats under amytal was found to reduce the glycogen content of the gastrocnemius by approximately 50 p.c. in 3 hours, to reduce the liver glycogen, and to produce hyperglycæmia.

The same dose of adrenaline caused a relatively greater mobilization of muscle glycogen in adrenalectomized rats, without producing marked hyperglycæmia. In thyroidectomized rats, hyperglycæmia was observed but there was little reduction of muscle glycogen.

Hypophysectomized rats gradually come to resemble thyroidectomized rats in that their muscle glycogen is not mobilized by adrenaline, while they resemble adrenalectomized rats in failing to display adrenaline-hyperglycæmia. Previous treatment with an anterior pituitary extract caused them to approach the normal in both respects, while administration of desiccated thyroid failed to restore the hyperglycæmic response.

We are indebted to Dr Selye for performing the hypophysectomies and to Mr L. W. Billingsley for the metabolic rate determinations.

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CONTRALATERAL EFFECTS FROM TENSION RECEPTORS IN SKELETAL MUSCLE

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(Received May 15, 1936)

Matthews [1931, 1933] has investigated the afferent discharge from tension receptors in skeletal muscle and has classified the latter as A1, A2, and B endings, identifying them respectively with the flower-spray endings of the muscle spindle, the annulo-spiral endings of Ruffini [1898] and the tendon organs of Golgi. The response of these receptors to active and passive tension was studied by him, and he suggested that the central effect of the discharge from the A1 endings was excitatory, from the B endings inhibitory, while uncertainty was expressed about that from the A2 receptors.

No direct experimental evidence has yet been offered for the central effects of these various centripetal streams, and the present authors submit the following experimental results on this problem. It appears impossible to isolate a particular group of proprioceptors and to study the reflex effects of the impulses set up by them. It is likely, however, that a complete section of a muscle at the junction of the muscle belly and the tendon, or the injection of a small amount of local anæsthetic into the tendon, will, if the centripetal streams from muscle spindles and tendon organs are opposite in effect, alter the central excitatory state succeeding active or passive tension in the muscle. It is obvious, of course, that both of the above procedures will affect some spindles as well as tendon organs, but it seems reasonable to assume that owing to the more intimate association of the spindle with the muscle belly [Ruffini, 1898] there will be a disproportionate denervation of the tendon organs.

We have investigated the effect of thus suppressing the tendon organ response on two crossed reflexes.

(*a*) The Philippson reflex in which stretch of a vasto-crureus muscle of one hindlimb results in contraction of the fellow muscle of the other side.

(*b*) The reflex [Cooper and Creed, 1927] where contraction of a vasto-crureus muscle evoked by electrical stimulation of the muscle, or its nerve, or motor roots leads to inhibition of postural contraction in the fellow muscle of the opposite side.

Methods

Decerebrate cats were used, and in all a series of twenty has been examined. In most cases one vasto-crureus was used as the exciting and the opposite vasto-crureus as the reacting muscle, but in four cases the opposite calf muscles were used as the reacting muscles. The exciting muscle was either a chronic preparation or an acute one. In the former case complete section of the muscle at the junction with the tendon was performed and the muscle resutured to the tendon and allowed to heal aseptically. Three months later when healing was complete and on examination the animal did not resent handling of the operated limb, decerebration was performed and proprioceptive reflex activity in the hindlimbs examined myographically. In the acute preparations section and resuture of the exciting muscle was carried out immediately after decerebration or the tendon infiltrated from the distal end with 1–2 c.c. of 1/1000 percaine solution. In every preparation both the exciting and the reacting muscles were isolated from extraneous reflex effects by section of the cutaneous nerves, and all the muscle nerves in both hindlimbs with the exception of those to the muscle being studied.

An isometric steel strip myograph was used and an optical recording system giving a magnification of 100. Rigid fixation of the animal was procured by the insertion of steel drills into the upper and lower ends of the femur (or tibia) on the side of the reacting muscle and into the pelvis on the side of the exciting muscle. These drills were then securely clamped to the steel frame on which the myograph was mounted. The reacting muscle was then attached to the myograph by means of a steel hook, the length of which could be readily adjusted to provide whatever initial tension in the muscle was considered desirable. Under these conditions when the nerve to the reacting muscle was sectioned maximal stimulation of the exciting muscle did not cause any deflection of the myograph record. The precaution was always taken of sectioning the rectus femoris on the side of the reacting muscle to avoid pelvic rotation.

Results

On resuturing the vasto-crureus after complete transverse section a large "bite" of the tendon was taken up in the suture to compensate for any subsequent stretching of the scar. This was in most cases completely successful, and from 2 to 3 months later the animal would walk with an

Fig. 1.

Fig. 2.

Fig. 3.

Figs. 1, 2 and 3. Photographs of a decerebrate cat in which the left vasto-crureus had been sectioned and resutured at the junction of the tension with the muscle belly 2 months previously. In Fig. 1, with the trunk and head disposed symmetrically in the supine position there is greater flexion of the knee joint on the operated side. Figs. 2 and 3 show the attitude of the left leg before and after tapping the patellar tendon once with a hammer (absence of the shortening reaction). Judging by the gait of the animal before decerebration and from manipulation there did not appear to have been any post-operative lengthening of the left vasto-crureus.

almost imperceptible limp. In two cases, however, a definite limp was present. In the early stages after section resuture considerable resentment was shown by the animal if the limb was handled or any attempt made to examine it. In the later stages the limb was used quite well, and during struggling movements it was impossible to detect any difference in the strength of the two hindlimbs. Under ether anæsthesia of appropriate depth the operated limb constantly exhibited greater flexion at the knee

joint (Fig. 1). The knee jerk was always present but was small in amplitude, and was not followed by a shortening reaction (Figs. 2 and 3) as on the normal side. Knee clonus appeared to be more easy to elicit on the operated limb side but was readily inhibited on gently handling the limb. After decerebration the same changes were seen, and although a definite knee jerk and myotatic reflex could be elicited in the operated muscle the lengthening reaction always appeared at a lower tension than normal.

Philippson's reflex.

Philippson [1905] described this reflex in chronic spinal dogs as follows: "forced flexion of the leg upon the thigh provokes in the opposite limb extension of the leg and foot, the thigh remaining flexed." It was

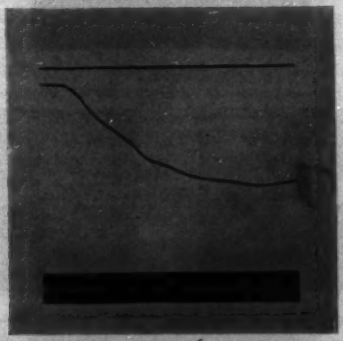

Fig. 4. Philippson reflex in the left vasto-crureus of a decerebrate cat. The right vasto-crureus (exciting muscle) had been operated on 2 months previously. All figures read from left to right. Increasing tension in the reacting muscle is indicated in the records by movement away from the horizontal base line of zero tension. Time marker records 100 d.v. per second.

later shown by Sherrington [1909] that this was a proprioceptive reflex set up by stimulation of tension receptors in the vasto-crureus. He also stated "it seems essential for eliciting the crossed reflex that the forced stretch of the knee extensor should be made when the muscle is exhibiting tonic contraction."

The reflex is indeed capricious and is best seen in decerebrate animals in which rigidity is marked.

We have found that the Philippson reflex may be abolished by section of the motor roots of the exciting muscle. It appears that this effect is

due to the absence of discharge from receptors stimulated by active contraction in the exciting muscle (A 2 or B), since in the reacting muscle, the postural contraction which was used as a background for examining the Philippson reflex, exhibited no evidence of partial inhibition. Such inhibition might be expected if the abolition of the Philippson reflex after section of the motor roots was due to inhibition from the exciting muscle arising in tension receptors which had been previously unloaded, partly or wholly, by the foregoing active contraction.

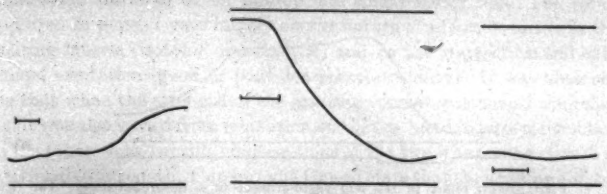

Fig. 5. Fig. 6. Fig. 7.

Fig. 5. Slow recruitment opening of a normal Philippson reflex in a decerebrate cat. Tracing of photographic record. Time marker (short horizontal line) =0·1 sec.

Fig. 6. Philippson reflex in the left vasto-crureus of a decerebrate cat. The right (exciting) vasto-crureus was a chronic preparation and had been operated on 3 months previously. The exciting muscle was paralysed immediately before making this record by intrathecal section of its motor roots.

Fig. 7. Same record as Fig. 6 taken 4 sec. later. Passive stretch of the exciting muscle is still continuing and the reflex response tends to persist.

In our chronic preparations (e.g. after tendon section and resuture) when both hindlimbs were completely denervated with the exception of the exciting and reacting muscles, crossed contraction was evoked in the normal muscle when the operated muscle was passively stretched (Fig. 4). Such crossed contraction could not properly be termed a Philippson reflex, since unlike the latter (Fig. 5) the contraction in this case persisted during the application of passive tension to the exciting muscle (Fig. 7). It thus appears that removal of the tendon organ discharge in the exciting muscle removes an inhibitory component from this reflex.

Further, after section resuture of the exciting muscle, section of its spinal motor roots no longer abolished the crossed contraction (Fig. 6), and the latter again continued for several seconds during the persistence of passive stretch in the exciting muscle. Similar observations were made (two experiments) after infiltrating the tendon of a normal "decerebrate"

vasto-crureus with percaine. When the exciting vasto-crureus was sectioned and resutured immediately after decerebration, marked crossed contraction followed stretch of the operated muscle (Fig. 8). The opening was more precipitous and the final tension much higher than in the normal Philippson reflex. Myographically the reflex now had several features in common with the crossed contraction set up by a nociceptive stimulus; and squeezing the operated muscle between the fingers elicited a crossed contraction showing the same rapid opening and even higher final tension than that which took place when the same muscle was passively

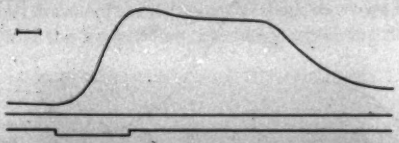

Fig. 8. Philippson reflex in the left vasto-crureus of a decerebrate cat. The right (exciting) vasto-crureus had been operated on (section-resuture) 30 min. previously. The signal indicates the approximate period during which tension was applied to the exciting muscle.

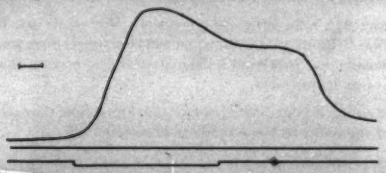

Fig. 9. Same preparations as in previous figure. Record shows reflex contraction of the left vasto-crureus by gently squeezing the belly of operated muscle on the opposite side.

stretched (Fig. 9). This "squeeze" reflex was not affected by section of the spinal motor roots of the operated muscle. But if it is admitted that this "squeeze" reflex in an acute preparation may be the result of nociceptive stimulation it would also be possible that crossed contraction in the chronic preparation is of the same character. Against this is advanced the fact that both in the intact and the decerebrate animal, manipulation and squeezing of the site of operation in the muscle did not arouse any reflex response other than that provoked by powerful pinching either of the skin or the operation site or elsewhere in the muscle belly.

The Cooper and Creed effect and contralateral soleus contraction

Cooper and Creed [1927] have described a reflex relaxation of one vasto-crureus provoked by active contraction of the fellow muscle of the opposite side. This reflex has been termed by us the Cooper and Creed effect. On repeating Cooper and Creed's work variable results were at first obtained by us. Sometimes relaxation was seen, sometimes no effect took place, and at other times contraction occurred in one vasto-crureus when the fellow muscle of the opposite side was made to contract by stimulation (faradic) of an appropriate spinal motor root. The result appeared to depend very largely on the nature of the contraction in the exciting muscle (isotonic or isometric) and on the state of central subliminal excitation (good or poor decerebrate rigidity). It was observed too that when the strength of the exciting current was varied the reflex result was also varied from relaxation on the one hand to pure contraction on the other, with varying combinations of the two when using stimuli of intermediate strength. Cooper and Creed state that the exciting current used by them was "weak", and apparently they did not vary its intensity.

This reflex was elicited in our decerebrate animals in most cases by stimulation of the peripheral end of the appropriate spinal motor root which had been isolated after laminectomy and then sectioned intrathecally. Usually two and sometimes three motor roots carry impulses to vasto-crureus, and it has been our practice to employ that root which innervated the greater fraction of the muscle (generally the last lumbar). If the exciting muscle was permitted to shorten when its motor root was stimulated it was constantly observed that very little or no reflex was produced. The more completely isotonic the contraction the less the response which was provoked. Finally, when the tendon of the exciting muscle was freed from its insertion and together with the adjacent belly of the muscle was dissected away from the investing deep fascia, maximal faradic stimulation of the cut motor root provoked either no reflex response or at the most an extremely weak inhibition amounting to a few grams only of the total postural tension originally present in the reacting muscle (Fig. 10). This agrees with Matthews' finding that no impulses are set up on contraction of a muscle with its tendon free. If the tendon of the exciting muscle was not freed from its insertion and the isotonic contraction permitted to extend the knee joint, the small mass imposed on the exciting muscle was usually sufficient to produce a wavering reflex response suggesting a struggle between weak excitation and weak inhibition in the motoneurone pool of the reacting muscles (Fig. 11).

With isometric contraction in the exciting muscle the nature of the reflex response was conditioned mainly by the strength of stimulation, while the intensity of the response was determined in great part by the initial tension in the exciting muscle. The reflex response provoked by

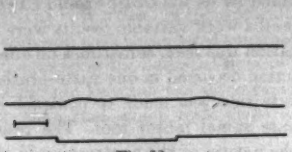

Fig. 10.

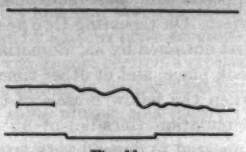

Fig. 11.

Fig. 10. Postural contraction in the left vasto-crureus during isotonic contraction (indicated by signal) of the opposite vasto-crureus (maximal faradic stimulation of the peripheral end of the cut motor root). The tendon of the exciting muscle had been cut free from its insertion and investing fascia dissected away from the muscle belly.

Fig. 11. Record obtained from a preparation similar to the preceding one with the exception that the maximal isotonic contraction of the exciting muscle was permitted to extend the leg.

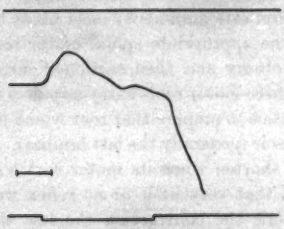

Fig. 12.

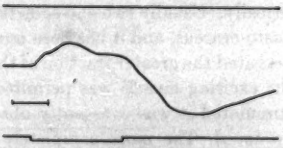

Fig. 13.

Fig. 12. Record of inhibition and rebound contraction in the reacting vasto-crureus produced by maximal isometric contraction of the exciting vasto-crureus (secondary coil at 18 cm.).

Fig. 13. Same type of reflex as in Fig. 12 produced in this case by submaximal stimulation of the exciting muscle (secondary coil at 25 cm.).

maximal stimulation of the cut motor root (Fig. 12) was initiated by partial inhibition of the original postural tension in the reacting muscle. During stimulation this inhibition was replaced by weak excitation which apparently gradually became prepotent. This can be seen in Fig. 12, which shows an unsteady rise of tension succeeding the original inhibition (the stimulus intensity being constant throughout). At the "off" of the stimulus rebound contraction was a constant feature, and the strength of

rebound was graded, within limits, by the strength of isometric contractions in the exciting muscle. The rebound after submaximal stimulation (Fig. 13) may be compared with that following maximal stimulation (Fig. 12). As the stimulus was made more and more submaximal the rate and amount of the tension changes became less until the reflex response was represented by small fluctuations of the original postural tension in the reacting muscle (Fig. 14). When the stimulus became minimal it was observed in several instances that the reflex now aroused consisted of a slow rise of tension of small amplitude followed by prolonged after-discharge (Fig. 15).

With stimuli up to 50 p.c. supramaximal the response was identical with the maximal response, and no tension changes were recorded with the supramaximal stimuli which might be the result of the high frequency

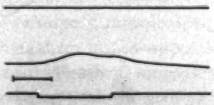

Fig. 14.

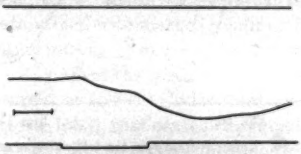

Fig. 15.

Fig. 14. With the secondary coil at 27 cm. the crossed effect is represented now by weak inhibition.

Fig. 15. With the secondary coil at 30 cm. there is often no response but on several occasions a small amplitude contraction was provoked.

A2 ending discharge which Matthews [1933] has detected under these conditions. It seems possible that this observation of Matthews may have been the result of an axon threshold effect, for in his preparations several centimetres of peripheral nerve were deprived of their blood supply for some hours; the threshold of the small diameter axons (more susceptible to temperature changes) proceeding to intrafusal muscle fibres of the spindles, might thus be raised considerably and require supramaximal excitation. In the preparations described here intrathecal section of the ventral spinal roots did not disturb the delicate vasa nervorum passing out to the mixed nerve trunk.

It was found that the responses described above were susceptible to changes in the temperature of the spinal cord, and it was necessary therefore in the intervals between successive root stimulations continuously to bathe the cord in warm Ringer's solution. In controlling our observations it was noted that the reflex effects could be completely abolished

by section of the dorsal roots of the exciting muscle, by anæsthetization of these roots with percaine, and by replacing the cut motor root on the stimulating electrodes by a moist cotton thread, one end of which was led down to the inner aspect of the arachnoid.

When the above experiments were repeated using a chronic preparation in which the section resuture operation had been carried out on the exciting vasto-crureus some months previously, a characteristic modification of the normal responses was noted. Although the initial inhibitory

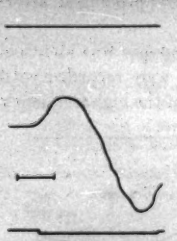

Fig. 16.

Fig. 17.

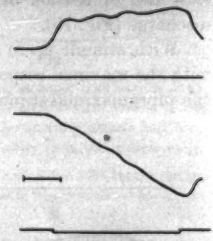

Fig. 18.

Fig. 16. Reflex response in the left vasto-crureus of a decerebrate cat when the cut motor root of the opposite vasto-crureus (chronic tendon organ preparation) contracted isometrically with the secondary coil at 28 cm. This record may be compared with the normal submaximal response in Fig. 13.

Fig. 17. Crossed response to submaximal isometric (secondary coil at 25 cm.) contraction of the exciting vasto-crureus after the injection of 2 c.c. of a 0·1 p.c. solution into its tendon.

Fig. 18. Record of left "soleus-gastrocnemius" thrust during submaximal isometric contraction of the right vasto-crureus. The soleus tension is recorded on the side of the time marker.

opening phase was still present it had become less marked and was immediately succeeded by a rapid rise of tension during the application of the stimulus (Fig. 16). In one experiment using percaine the normal responses were first recorded, and then 2 c.c. of 1/1000 solution were injected into the tendon of the exciting muscle. Within a few minutes the response was modified as described above, and the myograph record (Fig. 17) displayed the same rise of tension during stimulation. It was noted, too, after the above procedures that the reflex responses had lost the capacity to be varied qualitatively by varying the intensity of stimulation. With stimuli of different intensities the response was altered quantitatively, but, ranging from maximal to minimal, a marked rise of tension always took place during the application of the stimulus and, unlike the normal reflex, was not succeeded by rebound (cf. Fig. 16 with Fig. 14).

We have also observed (four experiments) crossed contraction of the soleus and gastrocnemius in response to active contraction of the exciting vasto-crureus. This reflex mainly involves soleus (Fig. 18), but a lesser amount of reflex tension occurred in gastrocnemius as well. This reflex did not exhibit the complex features of that in vasto-crureus and was usually a simple rise of tension commencing and ending with the active contraction in the exciting muscle. Its characters did not change after section resuture or anæsthetization of the tendon of the exciting muscle.

Discussion

It appears that the annulo-spiral endings of Ruffini (A 2) excite the Philippson reflex. The latter is abolished by section of the motor roots of the exciting muscle, so that the high threshold tendon organs (B) can play no part in exciting it. In a decerebrate animal with marked rigidity, after section of the motor roots of the exciting muscle, even excessive stretch of the paralysed muscle does not noticeably affect the tension of a postural contraction in the opposite vasto-crureus or soleus. Under these conditions it is known, moreover [Matthews, 1933], that centripetal impulses are being fired off at high frequency from all three types of receptors in the muscle. These centripetal streams from a paralysed extensor must neutralize one another in the contralateral extensor motoneurone pools. To these centripetal impulses there is, according to our present knowledge, only one available addition, namely, the still higher frequency discharge from A 2 endings which, according to Matthews' suggestion, is set up by contraction of intrafusal muscle fibres.

In the second place the experimental procedures described here, which it is believed decrease B discharge in proportion to A 2 discharge, both prevent the abolition of Philippson's reflex by motor root section of the exciting muscle and prolong the reflex contraction which now tends to persist with the stimulus. The temporal relations of this persistence have not been investigated but are probably related to adaptation in A 2 endings. These results appear to indicate the removal of considerable inhibition, presumably arising in B endings, thus leaving the A 2 endings more or less in charge of the reflex arc. It must be remembered too that the procedure described depresses myotatic contraction in the exciting muscle, which as stated previously is the essential factor in arousing the reflex in the normal decerebrate animal. If, however, it has at the same time removed contralateral inhibition (set up in ipselateral B endings) which would tend normally to neutralize A 2 excitation, it is

reasonable to assume that under the new conditions A2 discharge, set up by passive tension alone, is adequate to initiate crossed contraction, that the reflex will now tend to persist with the stimulus, and that with depreciated contralateral inhibition it is not necessary for further enhancement of A2 firing by intrafusal fibre contraction to take place. It is believed then that as far as the contralateral extensor motoneurone pool is concerned annulo-spiral endings are excitatory and B endings inhibitory.

We have not observed any contralateral effects which might be attributed to impulses arising in A1 endings. It may be emphasized that in the Philippson reflex the state of active contraction in the exciting muscle, which seems essential for eliciting the reflex [Sherrington, 1909], has been shown [Matthews, 1933] to "unload" the A1 endings. We have not observed any inhibition of postural contraction in the reacting vasto-crureus during forcible stretch of the exciting vasto-crureus paralysed by section of its motor roots (an operation which releases the A1 endings from "unloading"). No evidence has been obtained in our experiments to suggest that the A1 endings, in the vasto-crureus muscle at least, have other than an autogenous field of action on the motoneurone pool of the muscle in which they are situated.

Observations on the Cooper and Creed effect substantiate the foregoing conclusions concerning the effects of impulses set up in A2 and B endings on the contralateral motoneurone pool. Study of the records of this reflex obtained from a normal "decerebrate" muscle suggests that it is a mixture of inhibition and excitation in the motoneurone pool with the latter travelling in the train of the former and becoming effective only as contraction ceases in the exciting muscle. That it is the B endings which are responsible for contralateral inhibition is also suggested by the slight relaxation of postural contraction in the reacting muscle (Fig. 10). Under these conditions both A1 and A2 endings are "unloaded" by the shortenings, and the B endings record the very slight amount of total tension in the contracting muscle. After section resuture or anæsthetization of the tendon of the exciting muscle the reflex is modified so that some inhibition is lost and contraction (presumably excited from A2 endings) appears in the reacting muscle while the exciting muscle is still contracting.

Sherrington [1906] and Creed and others [1932] have already discussed in some detail the role of proprioceptive nerve endings in reflex walking, and the importance of rebound from inhibition has been pointed out. Previously, mention of such rebound has been confined to that which takes place in an extensor muscle after active contraction of an ipselateral

flexor. Rebound contraction of the quadriceps femoris group after active contraction of the fellow muscle of the opposite side must be an important synkinetic reflex in man during the change over from knee flexion to knee extension during walking (as in the right leg in Fig. 19). Grading of this rebound by the strength of contraction in the exciting muscle provides a mechanism for the automatic acceleration or deceleration of pace, voluntary excitation or inhibition in one spinal "half-centre" [Graham Brown, 1914] alone being necessary for mutually increased or decreased rebound in the two quadriceps femoris groups.

Fig. 19.

Fig. 20.

Fig. 19. Attitude during walking showing right leg about to pass into the extension phase and vice versa. Active contraction in the left quadriceps holds the fellow muscle of the opposite side inhibited (Cooper and Creed effect). Rebound from this will aid extension of the right knee. (Photo by super-speed cinematograph camera.)

Fig. 20. Attitude in which it is suggested that the soleus-gastrocnemius thrust comes into play in the left leg.

The importance of reflex contraction in the soleus-gastrocnemius group excited by active contraction of the contralateral quadriceps femoris is suggested by the attitude in Fig. 20. Soleus contraction, by plantar flexion at the ankle, compensates for the flexing knee joint and maintains propulsion and balance. Contraction of the gastrocnemius is a synergistic reflex supplementing plantar flexion of the ankle and flexion of the knee.

Summary

Observations were made of the contralateral reflexes excited by passive stretch and active contraction of one vasto-crureus muscle in decerebrate cats. These reflexes were also studied after decreasing the tendon organ discharge from the exciting muscle either by transverse

section of the muscle belly at its junction with the tendon or by anæsthetization of the tendon. After these procedures the Philippson reflex was no longer abolished by section of the motor roots of the exciting muscle and reflex contraction in the reacting muscle persisted during passive stretch of the exciting muscle. Active contraction of one vasto-crureus muscle provoked inhibition in its fellow muscle of the opposite side and this inhibition was succeeded as relaxation took place in the exciting muscle, by rebound contraction. This rebound was graded by the strength of contraction in the exciting muscle. Contraction of the calf muscles is excited by active contraction of the contralateral vasto-crureus. The significance of these reflexes in reflex walking is suggested.

The conclusion is made that the Philippson reflex is excited by impulses arising in the annulo-spiral endings of the exciting muscle and that impulses from the tendon organs of one vasto-crureus produce inhibition in the motoneurone pool of the fellow muscle of the opposite side.

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ON THE SPECIFIC VASO-DILATING AND PLAIN MUSCLE STIMULATING SUBSTANCES FROM ACCESSORY GENITAL GLANDS IN MAN AND CERTAIN ANIMALS (PROSTAGLANDIN AND VESIGLANDIN)

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(*Received May* 21, 1936)

Earlier investigations [Euler, 1934*a*, 1935*a*] have shown that extracts and secretions from the human prostate gland and seminal vesicles, as well as seminal fluid, greatly lower the blood pressure after injection into animals and, even in small amounts, stimulate the isolated intestine and the uterus. At about the same time Goldblatt [1933, 1935] independently observed similar actions for human seminal plasma. The active substance is different from hitherto known autopharmacologic active substances, including the "substance P" [Euler & Gaddum, 1931; Gaddum & Schild, 1934]. The latter substance, which is obtained from intestinal muscle and brain, stimulates the isolated intestine and lowers the blood pressure; it has recently been shown to be an albumose-like substance [Euler, 1936].

Biological actions of extracts from the accessory genital glands have been described several times before, but the older observations have received no great attention, probably because the experimental data do not permit of definite conclusions as regards the nature and the specificity of the active constituents.

Thus Japelli and Scafa found in 1906 that extracts of the prostate of the dog caused a rise in blood pressure in the dog on intravenous injection. This action is probably due to adrenaline, since Collip [1929] and Euler [1934*b*] have demonstrated the presence of this substance in considerable amounts in this and related glands. In rabbits a lowering of blood pressure was observed, but since knowledge of depressor substances was limited at that time, it is not possible to draw any conclusion with regard to the nature of the active substance or substances.

Thaon [1907] also found a rise in blood pressure, followed by a fall, in the rabbit, on injection of extracts from the prostate gland of the dog and the bull.

Camus & Gley [1907] found that intravenous injection of secretion from the internal prostate of the hedgehog (taken during a period of sexual activity) killed rabbits even in amounts of 0·3–0·4 c.c. per kg., the animals becoming severely dyspnoeic. Biedl [1916] has remarked in this connexion that the toxic effects were probably caused by intravascular clotting, especially as such secretions are known greatly to promote coagulation. Whether or not other active substances took part in the effect is not clear.

Götzl [1910] reports that press juice from human prostate glands (obtained from autopsy material or during operations), after injection into the ear vein of the rabbit, will kill the animal even in amounts of 2 c.c. Intravascular clots were found in these cases, and the blood was afterwards found to be permanently fluid.

The following year Dubois & Boulet [1911] found that water or glycerine extracts of the prostate of the dog, the bull and the sheep caused in most cases—twenty-seven out of thirty—inhibition of the isolated intestine of the dog, cat, rabbit and sheep, and also of the intestine *in situ*. This effect may, so far as is known now, be due either to adrenaline or to adenylic compounds, most probably the former. The same authors found in 1912 that water extracts of the prostate of the dog promoted contraction of the urinary bladder of this animal *in situ*, and believed that the prostate gland produced a hormone with this action. This conception [Dubois & Boulet, 1919] has not yet been confirmed.

Experiments with extracts from a fresh human prostate gland were carried out by Battez & Boulet [1913]. The material was obtained from a man of 20 years who had been executed. After intravenous injection of the extract in the dog, in an amount corresponding to 0·125 g. per kg., a strong depressor action was observed, accompanied by contraction of the bladder.

The first communication on a pharmacodynamic action of human seminal fluid originates from Kurzrok & Lieb [1931]. These authors found, on adding 1 c.c. of seminal fluid to a strip of human uterus suspended in a 100 c.c. bath, either an increase or a decrease in spontaneous movement or tone. Later, Cockrill *et al.* [1935] reported that most specimens of human semen caused inhibition of the isolated human uterus strip (0·4 c.c. semen in 100 c.c.). Some of the specimens caused contraction, but after they had stood for half an hour at *p*H 10 the effect was inhibition. At *p*H 11 all specimens were without action, and this was the case after short boiling. According to these authors, the active substance is dialysable, soluble in alcohol, and biuret negative. After the addition of atropine, the effect is inhibited, whereas it is enhanced by eserine. The observed action corresponds with about 10γ acetylcholine per c.c. of semen, which is a remarkably high content. In support of the opinion of the authors that the active substance is acetylcholine, it is stated that an inhibitory as well as a stimulating effect may be obtained from acetylcholine on the isolated uterus strip, and that both substances show similar properties as regards solubility and stability (both are destroyed in 30 min. at *p*H 11).

The pharmacodynamic effects of human seminal fluid and of secretions from the prostate and the seminal vesicles, found by Goldblatt and by Euler, manifested themselves most clearly in a strong depressor action in various animals and in a stimulating action on certain plain muscle organs. Goldblatt [1935] announces, however, that in his experiments the effect of human seminal plasma on the isolated uterus is probably caused by a substance different from the depressor and gut-stimulating one. This is not in conformity with the results to be reported in the present

paper, but there seems to be no doubt that, in spite of this, both investigators have been dealing with the same active substance.

With regard to the nature of the active substance, it is dialysable and soluble in water, alcohol, acetone, ether and chloroform. The substance is unstable at high temperatures, more so in alkali than in acid. Cataphoresis experiments have shown that the active substance has acid properties. In order to make reference more convenient, and since it is clear that this substance is different from other autopharmacologic substances, it has been preliminarily called "prostaglandin" [Euler, 1935*a*].

In extracts from the vesicular gland of the monkey (*Macacus rhesus* and others) a substance producing a marked fall in blood pressure in the atropinized rabbit could be demonstrated [Euler, 1935*b*]. In certain respects, however, this substance differs distinctly from prostaglandin. It has preliminarily been called "vesiglandin".

In the present paper the occurrence, properties, and biological actions of these new pharmacologically active substances are described so far as they are at present known.

Experimental results

Prostaglandin

(1) *Occurrence and demonstration of prostaglandin.*

During an investigation of various organ extracts for the purpose of finding the distribution of substance P, the observation was made that extracts from the human prostate had a pressor as well as a depressor action on the blood pressure of the atropinized rabbit. The pressor action could be referred to adrenaline, the occurrence of which seems to be widely spread in the accessory genital organs [Euler, 1934*b*]. After inactivation of the adrenaline by means of iodine at neutral or slightly acid reaction, the depressor action was left almost pure, though some loss probably occurred. This treatment also changed the inhibitory action on the rabbit's isolated gut into stimulation. Thus an effect was observed which in the respects mentioned agreed with the action of substance P, and since inactivation experiments pointed in the same direction it was assumed that the biological action of the extracts was due to the presence of substance P. On electrodialysing the active solutions, part of the activity was recovered on the cathode side whereas the anode solution was inactive [Euler, 1934*a*]. Later experiments have indicated, however, that, though a part of the action described is most probably due to the substance P, the greater part of it is caused by another substance. This

substance is carried by the electric current to the anode, but, owing to the development of free halogens, it is there rapidly destroyed, a fact which could be demonstrated with the purified substance (cf. p. 218). Similar effects to those observed in the extracts mentioned, deprived of adrenaline, were found in much higher degree in human seminal fluid and in secretion from the prostate and seminal vesicles obtained from autopsy material. Also in a few cases where the prostates were removed by operation the active substance could be demonstrated in the extracts. The remarkable pharmacodynamic action of human semen is shown by the fact that even 0·05 c.c., injected intravenously in the rabbit after atropinization, causes a prolonged lowering of the blood pressure. An obvious difference was observed in the action of substance P on the blood pressure, the action here being of relatively short duration. The rabbit's isolated gut was stimulated in a similar way as by substance P, i.e. the action consisted in a gradually developing increase in tone, frequently accompanied by a greater amplitude. The assumption that the active principle in prostatic extracts and in human seminal fluid is identical with that of substance P [Euler, 1934*a*] must be abandoned, however, since it has emerged during the purification experiments that the first-mentioned substance is not only soluble in alcohol (like substance P) but also readily soluble in acetone, and that it is not precipitated by ether from an alcoholic solution. The water-soluble part of the residue from the alcohol-ether filtrate shows the same actions as native seminal fluid. From this it is permissible to conclude (1) that human semen contains an active substance not hitherto known, with strong pharmacodynamic actions (prostaglandin), and (2) that this substance is almost solely responsible for the biological actions on the preparations mentioned.

(2) *Purification experiments.*

In most of the experiments human seminal fluid from various sources was used for the purification and test experiments, since this material was very active, on the one hand, and practically free from disturbing and biologically active contamination, on the other.

After the addition of 3–5 volumes of alcohol or acetone and a little hydrochloric acid—in order to bring the fluid to a slightly acid reaction—and filtering, the filtrate was evaporated under low pressure. After drying in the desiccator, the residue was laked with absolute alcohol and the alcoholic solution mixed with 3–5 volumes of dry ether. A precipitate resulted, which was removed by centrifugation from the clear yellowish alcohol-ether solution. The precipitate consisted to a considerable extent of

choline. The solution was evaporated and the residue extracted with water, leaving lipoids behind. The clear watery solution, which contained only a small amount of dry substance, was acidified to *p*H 4, if necessary, in order to make it stable.

From solutions in water the prostaglandin could be extracted by ether or chloroform but only when the solution was distinctly acid (about *p*H 3–4). From an alkaline watery solution practically no activity could be extracted with ether or chloroform. The extraction of the concentrated solutions of prostaglandin with ether was carried out in the following way. After the addition of hydrochloric acid to a reaction of *p*H 3–4, 10 volumes of ether were added and the whole shaken for half an hour. The separated ether phase was afterwards taken down to dryness and the residue laked with water. On drying this watery solution again, it was found that amounts less than 0·05 mg. were sufficient to cause distinct actions on the test preparations, i.e. on the rabbit's blood pressure or on the isolated gut of the rabbit.

In a series of experiments the partition coefficient between ether and water was determined at room temperature. The value of this coefficient in a number of biological tests was 0·07–0·10. This means that, in order to obtain a fairly complete extraction of the prostaglandin from a watery solution, it will be necessary to treat the solution several times with, say, 10 volumes of ether.

(3) *Solubility.*

Goldblatt has reported that the active substance is soluble in alcohol and acetone. Euler found that it was also, to a certain extent, soluble in ether and chloroform, as stated above. The prostaglandin is also soluble in concentrated acetic acid. The solubility in petroleum ether seems to be small. From the above it is clear that the solubility differs widely from that of the substance P, which can be precipitated by ether from an alcoholic solution.

(4) *Precipitation and adsorption.*

Hitherto all efforts to precipitate the prostaglandin by means of metal salts, strychnine or brucine have been without result. Phosphotungstic or reinecke acid did not precipitate appreciable quantities of the active substance from concentrated solutions. It appeared also from the experiments that the active substance was not readily adsorbed on precipitations. Special adsorption experiments with fuller's earth have corroborated this finding, in that 1 g. of fuller's earth in 15 c.c. of a solution

of prostaglandin, shaken intermittently for 2 hours, removes less than 20 p.c. of the activity of the solution. Treatment with fuller's earth, therefore, affords a useful method for the purification of solutions.

(5) *Stability.*

The stability was determined at varying *p*H at 100° C. and after 20 min. heating. The activity which remained after such treatment was determined on the rabbit's blood pressure, as well as on the rabbit's isolated gut. To acidify hydrochloric acid was used, and this was neutralized with sodium hydroxide and vice versa. The activity left after treatment is shown in Table I.

TABLE I

*p*H	Activity left after treatment at 100° C. for 20 min. p.c. of original
0	0
1–7	100
8·5	75
9·5	50
14	0

From the table it is evident that the stability is rather high, even as far on the acid side as corresponds to *p*H 1. On the alkaline side, however, the activity shows a rapid decrease.

(6) *Dialysis, electrodialysis, cataphoresis.*

The prostaglandin dialyses readily through cellophane, parchment or collodion. On electrodialysing active solutions, only traces of the activity could be recovered on examining the anode and the cathode fluid, though the middle chamber was deprived of most of its original activity. The type of electrodialysis apparatus used was a small specimen of the Ostwald type, both electrodes being continuously washed by a small current of distilled water. From these experiments it was clear that the active substance must have been destroyed, and since it will pass out at the anode, the acidity of the solution alone cannot be the cause of its destruction. It could be shown, however, that small amounts of free bromine rapidly destroyed the activity, and since free chlorine is regularly liberated at the anode it seems very likely that this is the cause of the failure of the active substance to appear in the anode solution.

In the cataphoresis experiments the Theorell technique [1934] was used, permitting an exact determination of the migration velocity at the same time. It was found that at an approximately neutral reaction

($pH = 6{\cdot}54$) the active substance went to the anode at a rate of $5{\cdot}4 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ volt^{-1}, whereas the fluid on the cathode side was inactive. After 4 hours' cataphoresis with 6 mA. at 20° C. of a purified solution of prostaglandin, the activities in the different segments (see Fig. 1) were determined on the rabbit's blood pressure and on the rabbit's isolated intestine. The activity of the contents in the numbered segments is given as a percentage of the original activity of the solution in Table II below.

TABLE II

Segment	−1 to −3	−4	−5	−6	4+ to +6	+3	+2	+1
Activity	0	30	60	85	100	100	30	15
	Cathode→				←Anode			

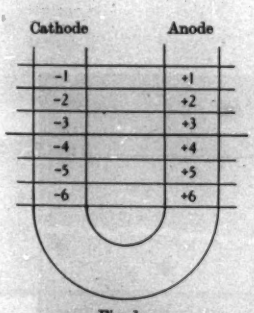

Fig. 1.

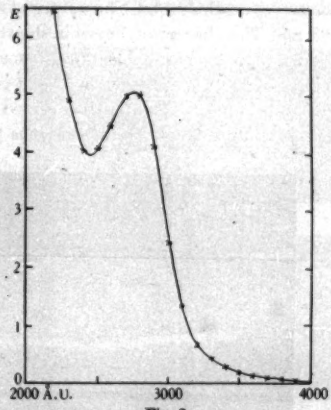

Fig. 2.

Fig. 1. Cataphoresis (Theorell).

Fig. 2. Absorption spectrum in ultra-violet of a purified solution of prostaglandin. Ordinate: $E = \frac{1}{c_1} \cdot \frac{1}{d} \cdot \log \frac{I_0}{I}$, c_1 meaning mg. purified substance per c.c. Abscissa: wave-length in Ångström units.

The prostaglandin thus has acid properties, which affords an explanation of the observation that only from acid watery solutions would ether and chloroform extract the active substance, whereas it was left behind in alkaline solutions. Furthermore, this property is a proof that the active substance cannot be mistaken for pharmacodynamically active substances already known in the body, since probably adenylic acid alone would come into question among these.

The absorption spectrum in ultra-violet of a purified solution of prostaglandin was also determined, Dr H. Theorell kindly doing this for me. The solution contained 2·25 mg. per c.c. and was prepared in the following way. The primary alcoholic extract was evaporated to dryness, the residue laked with a small quantity of absolute alcohol and the clear yellowish solution precipitated with 5 volumes of dry ether. The clear filtrate was dried and the residue taken up in water. This solution was slightly cloudy owing to the presence of lipoids, and was cleared with fuller's earth. The clear watery solution was again dried and submitted to the same treatment as outlined above. The final watery solution from the alcohol-ether filtrate residue was completely clear and colourless. It was active on the rabbit's blood pressure in an amount corresponding to 0·03 mg. The absorption curve is shown in Fig. 2. From the curve it is evident that the purified solution has a marked absorption band in the vicinity of 2750 Å. Whether this band is due to the active principle or to persisting impurities cannot be decided yet, though the high activity of the solution may be taken as evidence for the former assumption.

Fig. 3. Rabbit's blood pressure, urethane, atropine. 0·1 c.c. of a solution containing 2 mg. purified prostaglandin per c.c.

(7) *Action on the blood pressure.*

After intravenous injections of human seminal fluid or corresponding amounts of purified solutions of prostaglandin, a prolonged lowering of the blood pressure was observed in all animals tested (rabbit, cat, dog) (Fig. 3). After atropine the effect persisted. Of special interest is the long duration of the depressor action; in some cases as much even as

half an hour elapsed before the blood pressure had reached the original level. In this respect the known depressor substances, acetylcholine, choline, adenosine, histamine or substance P all produce an effect of fairly short duration; kallikrein alone has a prolonged action. This substance however shows no stimulating action on the rabbit's isolated gut and differs also in several other respects from prostaglandin.

(8) *Action on the heart.*

In the blood-pressure experiments, no definite influence was observed on the frequency of the heart or on the blood-pressure amplitude, as recorded with a membrane manometer. In some experiments a cardiometer was placed on the heart *in situ* but no definite changes occurred on

Fig. 4. Frog's isolated heart (Straub). *Prgl.* same preparation as in Fig. 2. Concentrations are shown in the figure. *x* denotes mechanical stimulation. *Ri*, frog's Ringer.

the injection of prostaglandin in amounts which lowered the blood pressure to half its normal value. The addition of corresponding amounts of prostaglandin to fluid perfused through the rabbit's isolated heart by Langendorff's technique did not cause any certain effect either on the frequency or on the amplitude of the contractions. These experiments make it highly improbable that the depressor action is of cardiac origin.

The effect of prostaglandin on the frog's heart was investigated with the Straub preparation. By means of a special arrangement, the heart could be set in communication with four different solutions, the change from one to another being made without loss of time. Frog's Ringer was

used at a pressure of about 5 cm. of water. For these experiments highly purified preparations of prostaglandin were used, of which even 20γ would cause a drop of about 20 p.c. in the rabbit's blood pressure. On changing the Ringer for prostaglandin-Ringer (P.R.) 1 : 500,000, an increase occurred in the heart rate from 24 to 35 as shown in Fig. 4. After adding P.R. 1 : 50,000 the effect was considerably accentuated, the frequency rising from 23 to 45 per min. At the same time a definite change in the type of the contractions could be observed which was characterized by a predominance of the systolic phase and an incomplete diastolic relaxation. In some respects the effect was similar to that caused by an excess of calcium. In one case a heart which had stopped when ordinary Ringer was used was started on simply changing the Ringer for P.R. 1 : 50,000, without any mechanical or other inducements. In this case the effect on the frequency and on the enforcement of the systolic phase was especially evident (Fig. 4 C).

(9) *Effect on the peripheral vessels in the frog.*

In a number of cases purified prostaglandin preparations were tested on the peripheral vessels of the hindlimbs of the frog (Laewen-Trendelenburg's preparation). *Rana temporaria* was used throughout and the pressure was 30 cm. water. After the frequency of the drops had become constant, the frog's Ringer was changed for P.R. 1 : 50,000. The Ringer was improved by adding about 10 p.c. of rabbit's heparine plasma to it, and this decreased the tendency to the development of œdema rather strikingly. Shortly after the change to P.R., vaso-dilatation occurred, showing itself in an increase in the number of drops (Table III).

TABLE III

No. of drops per 2 min.	Perfusion fluid
28	Frog's Ringer with 10 p.c. rabbit's heparine plasma
29	" " " " "
34	Same with prostaglandin 1 : 50,000
37	" " "
37	" " "
38	" " "
36	Frog's Ringer alone as above
34	" "
31	" "
26	" "

Though the vaso-dilating power of prostaglandin seldom exceeded the order of that shown in Table III, it could be demonstrated constantly.

(10) *Action on the isolated gut.*

The intestinal bath used was of the type described by Burn & Dale [1922], the capacity of the bath being 30 c.c. The suspension fluid was Tyrode, to which in most cases glucose was added (0·1 p.c.). The oxygenation was maintained by bubbling a mixture of 5 p.c. CO_2 in O_2 through the solution. The temperature of the thermostat was 38° C.

Prostaglandin caused a contraction of considerable duration on the isolated gut in all animals tested (rabbit, guinea-pig, rat, mouse, squirrel), as is seen in Figs. 5, 6, 9, 11, 12. The effect comprises partly an increase in tone, which sets in fairly quickly, though not so rapidly as in the case of acetylcholine or histamine, and partly an increase in the amplitude of the contractions. On the guinea-pig's intestine the difference in the time

Fig. 5. Guinea-pig's isolated intestine. *Prgl.* purified solution of prostaglandin; *hist.* histamine dihydrochloride.

course between the effect of histamine and that of prostaglandin was clearly evident, as shown in Fig. 5. In no case was the effect on the intestine test preparations from different animals affected distinctly by atropine. After the contraction had reached its maximum, it remained there for a relatively long time, in a way rather similar to that in the case of substance P. With regard to the frequency of the contractions no definite action could be observed.

(11) *Action on the intestine* in vivo.

The stimulating effect of prostaglandin on the gut could also be demonstrated *in vivo*. After subcutaneous injections of 5–10 rabbit doses, one rabbit dose causing a decrease of about 25 p.c. in the blood pressure, in white mice of 20–25 g. weight, a strong effect on the peristalsis of the intestine could be observed in a few minutes, resulting in the passage of semifluid fæces.

(12) *Action on the isolated uterus.*

Prostaglandin was found to stimulate the isolated uterus or strips of uteri of all animals investigated (cow, rabbit, guinea-pig, rat) and also strips of human uteri. The experiments with isolated uteri were performed in the same way as those on the intestine.

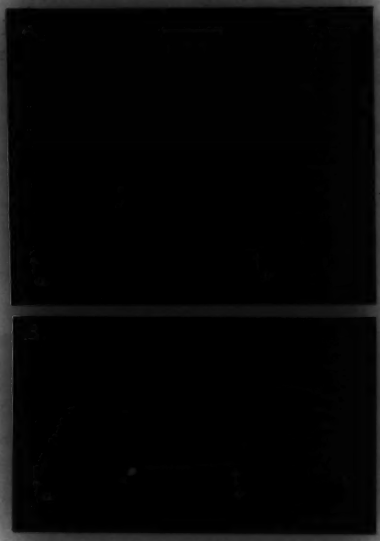

Fig. 6. A, rat's isolated uterus. *a*, 0·22 c.c. secretion from human semina vesicle (autopsy material). *b*, extract of 1·4 g. human prostate (autopsy material). B, rat's isolated intestine. *a* and *b* as in A.

Figs. 6 and 7 show the effect of prostaglandin on uterus preparations of the rat and the rabbit. On the virgin rabbit's or guinea-pig's uterus the effect was small, however, even when rather bigger doses were employed (Fig. 7).

It might be thought that the effect observed on the guinea-pig's uterus was at least partly due to contaminating histamine. That this was not the case could be shown in several ways: (1) the histamine equivalent of the purified solutions used—as determined on the cat's blood pressure —was of a much smaller order; (2) the effect was absent or very weak on the virgin uterus; (3) the effect disappeared after boiling for 20 min. in

normal acid, and this is not the case with histamine. The otherwise disturbing contamination of histamine and choline was chiefly avoided by extraction with ether, which leaves practically all of these substances behind. It could also be shown that, on partial inactivation by means of acids or alkali, the actions on the blood pressure, on the isolated intestine and on the isolated uterus always showed very close agreement. The opinion of Goldblatt [1935] that the active substance in human seminal plasma has no action on the uterus, but that the effect observed is due solely to histamine, is not supported by the present investigation.

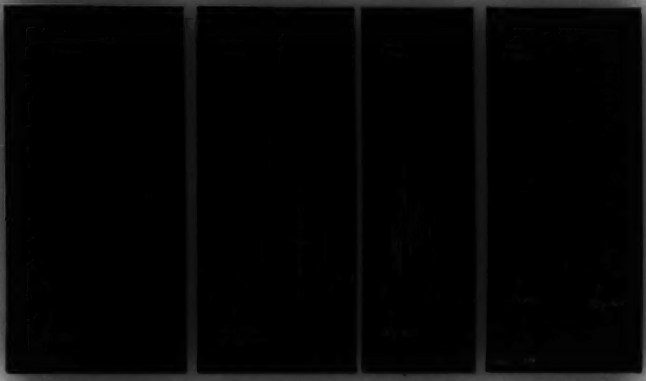

Fig. 7. A, rabbit's isolated intestine, purified solution of prostaglandin. B, rabbit's isolated uterus, same amount of prostaglandin. C, rabbit's isolated uterus, 50γ histamine dihydrochloride. D, rabbit's decidualized uterus (virgin), weak response to big dose of prostaglandin and histamine.

On strips of human uteri a stimulating effect was regularly found, which confirms some of the observations made by Kurzrok and his co-workers [1931, Cockrill *et al.* 1935] and is illustrated in Fig. 8. The last-mentioned authors found in many cases an inhibition, however, but in our experiments this was never the case. Strips of human uteri were tested in five cases. The operation material was kindly placed at my disposal by Prof. A. Westman, Lund. The strips of uteri used in my experiments were in most cases taken from the circular fibres arranged round myomatous nodes. The test pieces were about 4 cm. long and some millimetres thick. In one case a piece consisting of longitudinal fibres was tested, with the same result.

The effect on the human uterus consisted mostly in an increase in the contractions, but in a few cases also a marked increase in the frequency of the contractions could be observed. In the latter case the amplitude of the contractions was not influenced (apparently it was maximal). In one single case only was inhibition observed on a strip of a cow's uterus, such as Kurzrok and co-workers have described for human uteri. The piece on which inhibition was found consisted of longitudinal fibres, whereas another preparation of circular fibres reacted with a contraction. The uterus in question contained a fœtus the size of a hazel nut. In order to try to determine whether the inhibition reaction of the longitudinal

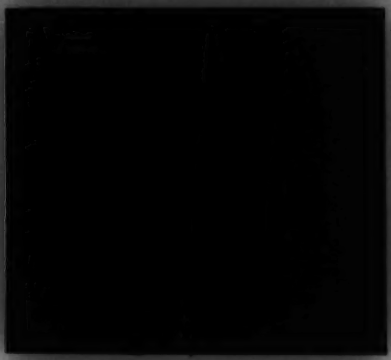

Fig. 8. Longitudinal strip of human uterus, grav., mens. IV. 0·1 c.c. of human seminal fluid.

piece was specific or not, choline and histamine were added to the bath, both substances causing a definite contraction, however. The inhibitory action of prostaglandin in this case was registered repeatedly with the same result, but with other (non-pregnant) preparations a similar reaction was never found, the only answer to prostaglandin being contraction.

(13) *The occurrence of prostaglandin in animals.*

After the demonstration of this new active substance in secretion and extracts from the prostate and seminal vesicles in man, its occurrence in corresponding organs of various animals was searched for. The following organs were investigated and tested for the presence of prostaglandin: prostate of monkey, horse, pig, dog, cat and rabbit; the vesicular gland

of monkey, bull, sheep, pig; the seminal vesicles of guinea-pigs; Cowper's glands of pig and sheep, and also fresh semen from bull and horse (I am indebted to Dr E. Åkerblom for these samples). Of the preparations mentioned, which were tested either in their native condition when this was possible, or in suitably prepared extracts, only the secretion from the vesicular gland of the sheep was found to exert a strong and typical prostaglandin effect on the rabbit's blood pressure and on the isolated gut (Fig. 9). As already stated, the accessory genital glands are relatively rich in adrenaline, but after inactivation of the adrenaline the extracts

Fig. 9. A, rabbit's blood pressure, urethane, atropine, 0·1 c.c. of secretion from sheep's vesicular gland. B, same amount added to rabbit's isolated intestine. C, *c*, prostaglandin extracted with ether from extract of human seminal fluid; C, *d*, ether extract from 0·1 c.c. of secretion from sheep's vesicular gland. (C, rabbit's isolated intestine.

too showed a prostaglandin effect. The identification of the active substance of the vesicular gland of the sheep with prostaglandin is founded on the following facts: (1) the quantitative relations with regard to the action on the rabbit's blood pressure, the isolated intestine, and on the isolated uterus of the rabbit, agreed with the action of purified prostaglandin prepared from human semen; (2) the active substance is inactivated in the same way by acids and alkali; (3) the active substance from the sheep can be extracted with ether in a similar way as for prostaglandin. These facts are stressed, since it is astonishing that the active substance in question, apart from being found in the secretion of the prostate and seminal vesicles of man, appeared only in the secretion of the vesicular gland of the sheep, and not in the corresponding secretions from the bull, the horse, or the pig. The assumption that the specific substance is produced perhaps only at the time when the animals are on heat is opposed

by the fact that none of the substance was found in the semen of the bull or the horse. In a number of cases the secretion of the vesicular glands of the pig was investigated, where these glands contained more than 600 c.c. fluid, the animals being in a period of sexual activity.

On the other hand, it cannot be doubted that the amount of the active substance in man, as well as in the sheep, varies greatly with fertility age. Thus only small quantities were found in prostates from children or old persons (autopsy material) whereas the same organs or the secretion from men between 20 and 50 were comparatively rich in the same. Similar effects were observed in the sheep, and furthermore the periods of heat showed themselves clearly in the size and the amount of secretion in the vesicular gland. The amount of prostaglandin seemed to follow the amount of secretion.

Whether prostaglandin is totally absent or present only in minute quantities in the secretions of the other animals investigated cannot be decided. In the secretion of the prostate of the rabbit an effect was sometimes observed, but whether this was due to a substance of the type of substance P or prostaglandin is uncertain. At any rate the amount of this substance prevails with a broad margin in the secretions of man and the sheep.

Vesiglandin

(1) *Occurrence and properties.*

With extracts from the vesicular gland of a monkey, *Macacus rhesus*, the observation was made [Euler, 1935*b*] that they caused a marked fall in the blood pressure of the rabbit and other animals. This action, like the action of prostaglandin, was of long duration and was not affected by atropine. The source of the two active substances being similar, it was thought conceivable that they were identical, but the behaviour on the isolated intestine and uterus, as well as inactivation experiments later on, showed that this could not be the case. It thus became clear that the substance from the monkey was different from others and, in order to facilitate reference, it has been called vesiglandin.

Vesiglandin shows a number of properties similar to those of prostaglandin, not only with regard to the effect on the blood pressure but also with regard to solubility and chemistry. It is soluble in water, alcohol, acetone, and is not precipitated by dry ether from an absolute alcoholic solution. It may be extracted by ether from the dry substance or from an acid watery solution. It is dialysable through cellophane. It was not precipitated by phosphotungstic or reinecke acid, nor by silver or lead salts.

On cataphoresis vesiglandin, like prostaglandin, goes to the anode. In electrodialysis experiments no activity was recovered, which is explained by the fact that vesiglandin, like prostaglandin, is rapidly destroyed by the free halogens at the anode.

It might be assumed, however, that, since the two substances have so many similar properties, vesiglandin might be a component of prostaglandin, the former substance having only a depressor action. Against the assumption that prostaglandin is composed of one depressing substance (vesiglandin) and one intestine-uterus-stimulating one, definite evidence is afforded by the fact that the two actions of the prostaglandin are inactivated at the same rate, which is markedly different for vesiglandin, as is shown in Table IV.

TABLE IV. Inactivation of vesiglandin at 100° C. for 20 min. at different reactions

*p*H	Activity left. p.c. of original
0	0
1	10
4	100
7	50
8–8·5	40
9–9·5	20
14	0

From the table it is evident that vesiglandin shows a marked maximum of stability at *p*H 4. When compared with prostaglandin, the stability curve shows definite differences, the stability of prostaglandin being greater, especially at *p*H 1, where none of the activity of the latter substance was destroyed. As practically all the vesiglandin is destroyed at this *p*H under the conditions mentioned, this difference may well be used for discriminating between the two substances. At *p*H 7, too, vesiglandin is definitely less stable than prostaglandin. The biological titration was made on the blood pressure of the atropinized rabbit, and prostaglandin was tested simultaneously in the same way and after the same treatment in order to strengthen the evidence with regard to the difference of the stability of the depressing effect of the substances. The results thus obtained with prostaglandin agreed very well with those previously found.

The material for the preparation of vesiglandin was partly the vesicular glands of monkeys, in extracts of which the new substance was detected, and partly semen from monkeys.[1] Also in extracts from the prostate glands of monkeys (chiefly *Macacus rhesus*) the active substance

[1] On this occasion I wish to thank Prof. Carl G. Hartman, Baltimore, who kindly supplied specimens of monkey's semen, which were taken up in alcohol.

could be demonstrated. In the case of prostaglandin, the secretion of the glands and extracts of them yielded the same active substance, and the same was so for vesiglandin. From this it may be concluded that the activity of the extracts was due to the secretion products of the glands. It follows also that, apart from the vesiglandin, no other pharmacodynamically active substance of importance is present in these glands when tested on the rabbit's blood pressure or the rabbit's gut. The conditions in the monkey are thus rather similar to those in man and in the sheep, with the difference that the active substances show distinct differences biologically as well as chemically.

(2) *Biological actions.*

Of the pharmacodynamical actions of vesiglandin, the effect on the blood pressure of the atropinized rabbit has already been mentioned.

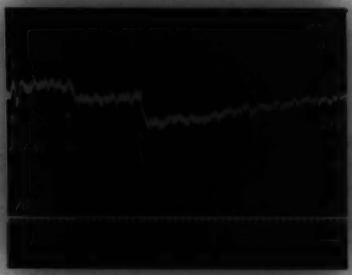

Fig. 10. Rabbit's blood pressure, urethane, atropine. *a*, extract of 4 mg. fresh vesicular gland of *Macacus rhesus*; *b*, extract of 8 mg.

This very much resembles that caused by prostaglandin in that it is of long duration. A more detailed analysis of the effect could not be carried out, as the supply of material was very limited. Fig. 10 shows the effect of vesiglandin on the rabbit's blood pressure after atropine. The amounts of fresh gland which corresponded to the preparations injected intravenously were in this case 4 and 8 mg. respectively. On the injection of an extract corresponding to 20 mg. of the fresh vesicular gland, depression of about 50 p.c. of the normal blood pressure was repeatedly observed.

On pieces of rabbit's isolated gut such amounts as were highly active on the blood pressure mostly caused either a very weak stimulating action or no action at all. Fig. 11 shows the effect of vesiglandin on the isolated

gut of the squirrel (which reacts like the rabbit in this respect) in comparison with that of a dose of prostaglandin having the same depressor effect.

It seemed to be of special interest to compare the effect of vesiglandin with that of prostaglandin on the uterus. Active preparations were

Fig. 11. Squirrel's isolated intestine. Purified solutions of prostaglandin and vesiglandin having equivalent actions on the blood pressure of the atropinized rabbit.

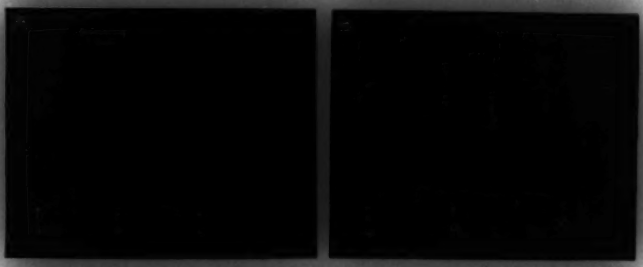

Fig. 12. A, rat's isolated uterus. B, upper tracing, rat's uterus, lower tracing, rat's intestine. *a*, 10γ histamine dihydrochloride; *b*, extract of 13 mg. vesicular gland of *Macacus rhesus*; *c*, depressor equivalent amount of prostaglandin; *d*, same as *c*, treated with NaOH *N*/1 for 5 min. at 100° C.

tested on the isolated uterus of the rabbit, the rat and the guinea-pig. On the rabbit's isolated uterus a stimulating action could be observed in some cases, but this was only about one-tenth of that of prostaglandin, comparing doses with the same depressor action. The action on the uterus agreed well with that on the intestine in these cases, and it is conceivable

that the preparations of vesiglandin contain a small amount of prostaglandin, a suggestion which does not seem unlikely in view of the fact that both substances show certain resemblances in biological and chemical respects and also with regard to their origin. Fig. 12 shows the effect of equivalent depressor amounts of vesiglandin and prostaglandin on the isolated intestine and uterus of the rat. On the guinea-pig's uterus only an indistinct effect was observed.

From these observations it follows that, though vesiglandin has effects on the blood pressure similar to those of prostaglandin, its action on certain plain muscle organs is either small or absent.

TABLE V. A schematic survey of some properties of certain biological "depressor substances"

		Solubility				Maximal stability at 100°, pH	(Rabbit) Biological actions			
								Isolated		
Substance	Dialysability	Water	Alcohol	Acetone	Ether		Blood pressure	Jejun.	Uterus	Influence of atropine
Kallikrein	−	+	−	−	−	*	−†	0	0	0
Adenosine	+	+	−	−	−	7–14	−	−	+	0
Acetylcholine	+	+	+	(−)	−	4	−	+	+	+
Histamine	+	+	+	(−)	−	0–7	(+)	0	0	0
Substance P	+	+	+	(+)	−	1–7	−	+	+	0
Prostaglandin	+	+	+	+	+	1–7	−†	+†	+	0
Vesiglandin	+	+	+	+	+	4	−†	0	0	0

* Unstable at 100°. † Prolonged action.

In Table V some properties of a number of biologically occurring "depressor substances" are summarized in order to show some characteristic differences with regard to solubility, stability and biological actions.

DISCUSSION

The occurrence of two substances, here called prostaglandin and vesiglandin, which were unknown up to a few years ago, and which exert remarkable biological actions in small amounts, in the secretion of accessory genital glands of man and certain animals, must be considered to point to a physiological function of these substances. From the experience available at present, it seems that the occurrence in these organs is specific, as it has not been possible to demonstrate a similar action in reasonable quantities of other organs. From the location of the active substances in the organism, the possible significance for the sexual activity as a whole or in certain details suggests itself. The chief actions observed, vaso-dilatation and strong contraction of plain muscle, are both intimately connected with the sexual functions. In spite of the fact that a physiological function of the substances is merely hypothetical as yet, it

may be suggested, on account of the presence of the substances in the secretion of certain genital glands, that they act as a stimulus for the emptying of these glands when the substances have accumulated sufficiently. It is perhaps also conceivable that after being produced in the gland the substances are absorbed into the blood, and act from there in some way or other. In both cases the substance would act as a sort of automatic regulator for the emptying of the glands. The testing of these possible physiological functions is rather difficult, however, especially in view of the important psychical factors which to a great extent regulate the sexual functions. In favour of the idea of a chemical stimulation by the secretion products it might be mentioned that the emptying of these glands is known to decrease greatly for some time their tendency to contract until the new-formed secretion products have accumulated sufficiently again. It is, however, difficult at present to see why such a substance should occur only in certain animals and in man.

Summary

In secretion and extracts from the prostate and seminal vesicles of man and the vesicular gland of the sheep a pharmacodynamically highly active substance, prostaglandin, has been demonstrated and described in respect of certain biological and chemical properties.

The substance which is soluble in water, alcohol, acetone, and, under certain conditions, in ether and chloroform, is very stable at *p*H 1–7 but is readily destroyed in normal acid and alkali, and also by free halogens.

In cataphoresis experiments prostaglandin goes to the anode with a migration velocity of $5{\cdot}4 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ volt^{-1} at *p*H 6·54.

It dilates the vessels of the hindlimbs of the frog and lowers the blood pressure of the rabbit, the cat and the dog.

It does not greatly affect the mammalian heart, but causes an increased frequency and predominance of the systolic phase of the frog's isolated heart.

The activity of the intestine of various animals is increased *in vitro* and *in vivo*.

The activity of the isolated uterus of various animals is enhanced, and this is also true for strips of human uterus.

The biological actions are not abolished by atropine.

Prostaglandin could not be traced in ejaculates from the horse or the bull, nor in secretions from the pig.

In semen or extracts from the vesicular gland or the prostate of the monkey (different kinds) another highly active substance was demonstrated (vesiglandin).

Like prostaglandin, vesiglandin is acid in character and shows similar properties with regard to solubility. It is, however, less stable in acids and alkali than prostaglandin.

Vesiglandin causes a lowering of the blood pressure of the atropinized rabbit, but shows no or only a weak effect on the isolated intestine of the rabbit or guinea-pig.

The possible function of these substances in the regulation of the expulsion of secretions from the accessory genital glands is briefly discussed.

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THE TECHNIQUE OF SUPRARENALECTOMY IN THE GUINEA-PIG AND THE SURVIVAL PERIOD AND CAUSE OF DEATH OF GUINEA-PIGS AFTER BILATERAL SUPRARENALECTOMY

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(*Received June* 8, 1936)

Part I. Technique of suprarenalectomy

Because of its difficulty suprarenalectomy in the guinea-pig is usually performed by morcellement. After morcellement, however, the suprarenals are unsuitable for examination and their complete removal cannot be verified; the necropsy performed for this purpose is futile because fragments left behind at operation may disappear or become unrecognizable before death. Clean surgical excision of the unbroken glands is free from these objections.

The following technique, based on a systematic anatomical and radiological study of the field of operation, makes possible the excision of each suprarenal whole and undamaged.

Anæsthesia

The guinea-pig is made drowsy by the following freshly made solution: urethane 25 g., sodium chloride 0·85 g., distilled water to 100 c.c. The dose, urethane 1 mg. per g. body weight, is injected subcutaneously at the back of the neck. The administration of ether vapour is begun just before the skin is incised.

Right suprarenalectomy

Right suprarenalectomy, being more difficult than left, is described in full.

(1) Shave the skin, clean it with methylated spirit and make a 2–3 cm. incision over the last intercostal space parallel to the ribs, ending about 1 cm. from the mid-line posteriorly.

(2) Open the abdomen throughout the length of the last intercostal space.

(3) Crush and divide the penultimate rib with artery forceps near the outer border of the lumbar muscles.

(4) Lift the kidney into the wound and retract it caudad.

(5) Free the suprarenal completely from the tissues of the posterior abdominal wall.

(6) Retract the kidney posteriorly, exposing the anterior surface of the suprarenal.

(7) Gently separate the superior border of the suprarenal from the liver, exposing the suprarenal vein at the supero-internal angle of the suprarenal.

(8) Pass a small aneurysm needle round the suprarenal vein from without inwards to avoid the inferior vena cava, thread the needle, and ligate the suprarenal vein.

(9) Divide the suprarenal vein distally to the ligature and separate the suprarenal first from the inferior vena cava then from the kidney.

(10) Without ligating, divide the suprarenal arteries entering the gland at its infero-internal angle, freeing the suprarenal.

(11) Stitch the abdominal parietes in one layer with 000 catgut and the skin with no. 90 thread.

Left suprarenalectomy

The procedure is similar except that easier access makes it unnecessary to divide the penultimate rib. The suprarenal is retracted cephalad to separate the suprarenal vein from the left renal vein. A small vein running from the infero-internal angle of the gland to the left renal vein is ligated when present.

Part II. Survival period and cause of death

Earlier workers found that guinea-pigs deprived of both suprarenals lived as a rule only a few hours. Much longer survival periods were observed by Rogoff. His results are given in Stewart's [1924] review of the literature. The second gland was removed either at the same sitting as the first or 2–3 weeks later. The surgical technique is not described. Of the twenty guinea-pigs studied, fourteen died within 7 days, six from 8 to 28 days, after removal of the second gland. The only later work seems to be that of Oefelein [1932]. He removed both glands at one sitting by morcellement from "etwa 50" guinea-pigs. They died as a rule 7–8 days later.

These survival periods throw no light on the cause of death. This can be elucidated only by comparing the survival periods after bilateral suprarenalectomy with those after control operations, as in the work to be described.

Material and methods

The material consisted of 189 guinea-pigs, all except four being males. The guinea-pigs were of various colours and their mean body weight immediately before operation was 396·6 g., the standard deviation of the distribution being 69·8 g. The diet, both before and after operation, was: dry oats and bran, greens, carrots, and water. The surgical technique is described in Part I. Following current practice with other species, bilateral suprarenalectomy was performed at two sittings. Right suprarenalectomy, as the more difficult operation, was performed first. The left gland was removed after 7 days, this interval being chosen for convenience. The left blank operation consisted in freeing the suprarenal from the surrounding tissues without actually removing it or endangering its blood supply. At the end of every operation the guinea-pig was placed in a room at about 24° C. overnight and was then returned to the animal house. Post-mortem examinations were made on all guinea-pigs except those alive 39 days after the blank operation. No accessory cortical tissue was found, and the only sign of disease observed was bilateral pneumonia in a guinea-pig found dead 2 days after removal of the left suprarenal.

Results

The immediate post-operative appearance and behaviour of the guinea-pigs were independent of the nature of the operation. Guinea-pigs that bore any operation well were eating, drinking, and showing

TABLE I. Survival periods of guinea-pigs after various operations

Nature of operation	No. operated on	No. found dead on day							P.c. dead within 7 days
		1	2	3	4	5	6	7	
LS	78	5	1	1	0	1	0	0	10·3
RS	111	10	9	4	2	2	1	3	27·9
RS + LB	20	0	2	2	0	0	1	0	25·0
RS + LS	50	8	5	4	16	11	3	3	100·0

LS = left suprarenalectomy, RS = right suprarenalectomy, LB = left blank operation.

Fate of guinea-pigs not found dead within 7 days

After LS. One found dead on 11th, one on 21st day, one in 2 months; all others killed 7–21 days after LS.

After RS. Seven days after RS, LB was performed on twenty and LS on fifty, the remaining ten being discarded.

After LB. One found dead on 12th, one on 21st day, others still alive and well 39 days after LB.

normal activity within 24 hours. All, however, showed ruffling of the hair; this condition decreased later but rarely disappeared entirely. After recovering from the operation, guinea-pigs from which the second suprarenal was removed remained well until within 24 hours of death.

The survival periods after various operations are shown in Table I.

Discussion

Table I shows that after the control operations most deaths occurred during the first 3 days, but that after bilateral suprarenalectomy most deaths occurred on the fourth and fifth. The results therefore indicate that after the third day following removal of the second gland, suprarenal insufficiency was a more important lethal factor than trauma.

Summary

1. A technique for excising the guinea-pig suprarenal whole and undamaged is described.

2. The left suprarenal was removed 7 days after the right from fifty guinea-pigs; all died within 7 days, the daily mortality being highest on the fourth and fifth.

3. The results of control operations show that after the third day following removal of the second gland, suprarenal insufficiency was a more important lethal factor than trauma.

We are indebted to Prof. S. L. Baker for criticism. The cost of the work was borne in part by the University committee on grants in aid of medical research.

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612.816.1

MULTIPLE RESPONSE TO CONSTANT CURRENT IN FROG'S MEDULLATED NERVE

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(*Received June* 22, 1936)

According to Hill's [1936] theory of electric excitation a constant current applied to an excitable tissue rapidly builds up at the cathode a "local potential" V which is maintained throughout the passage of the current: excitation occurs if and when V becomes greater than U, the "threshold". It would appear that excitation should continue to occur until the threshold U, rising at a rate determined at any moment by the value of V at that moment and by the time constant λ of "accommodation", becomes greater than V once more. During a certain time T, therefore, V will be greater than U (see Fig. 1 and Hill, 1936, Figs. 1 and 3), and throughout this time repetitive response might be expected to occur, at intervals determined by the refractory period. The duration of the repetitive response would clearly be greater the greater, according to theory, the value of λ, i.e. the slower the accommodation process. It will be shown below that $T = \lambda \log_e I/I_0$, where I is the strength of the applied current and I_0 is the rheobase.

The same repetition of response, according to theory, should occur at the anode after breaking a constant current through a nerve [see Hill, 1936, Figs. 2 and 4].

Experiments have been made to ascertain whether the theory can be applied in this way to the phenomena of multiple response in frog's medullated nerves. In crab's nerve [Katz, 1936] repetitive response is very obvious, and—according to the theory—is a natural consequence of the slowness of accommodation.

Method

The experiments were made during January and February. Sciatic nerves and gastrocnemius-sciatic preparations of Hungarian *Rana esculenta* previously acclimatized to different temperatures (between 0

and 19° C.) were used; they were soaked in Ringer's solution of various calcium contents. Most experiments were made at room temperature, a few at 2–5° C. A paraffin-wax chamber was used with non-polarizable calomel half cells connected with the nerve by wooden strips saturated

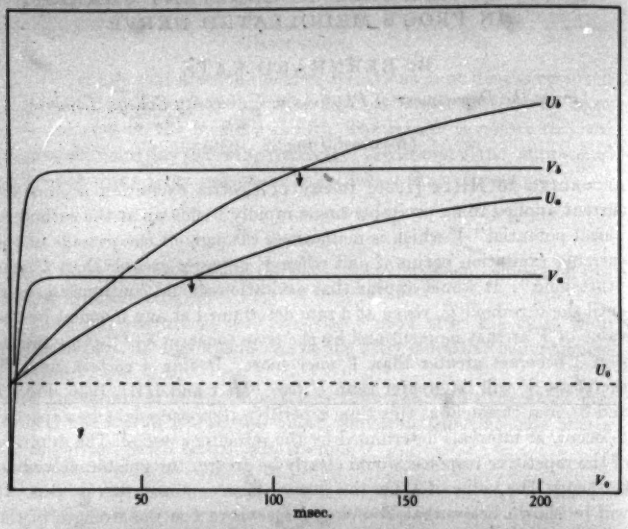

Fig. 1. Diagram illustrating threshold changes after closure of constant current. "Local potential" V and "threshold" U are represented by separate curves [cf. Hill, 1936]. Constant current of (a) twice and (b) 3 times "rheobasic" strength. Curves calculated, according to Hill, for $k=2{\cdot}5$ msec., $\lambda=100$ msec. In order to illustrate the rise of threshold and of local potential on the same time scale, a much too large k has been chosen. (A usual value of k would be 0·5 msec.) The end of the interval T of "maintained excitation" is shown by arrows.

with Ringer's solution; strings of wool soaked in Ringer's solution connected the muscle with lead-off electrodes. The electric response of nerve, or muscle, during the passage of continuous current of various intensities through the nerve, was recorded by a three-stage capacity-coupled amplifier and Downing [1936] oscillograph. In the intervals between two stimuli, in order to reverse any after-effects, shocks of equal strength and opposite direction were sent through the nerve. In

some experiments the response of nerve, or muscle, to repetitive nerve stimulation was recorded (condenser discharges of $RC = 0{\cdot}1$ msec. at various frequencies, or constant current pulses of 10 msec. duration at about 30 per sec.). The time factor λ of accommodation was determined with exponentially increasing currents [Solandt, 1936].

Results

(1) *The response of "cold frogs'" nerves to constant current.* The frogs used during these experiments had acclimatized incidentally to a rather low temperature. The isolated sciatic nerves were soaked for 2–6 hours

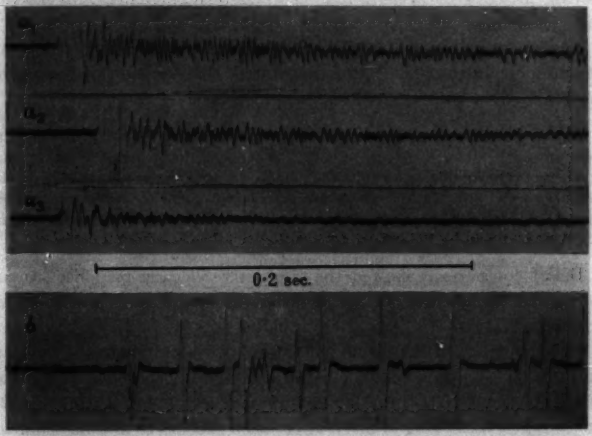

Fig. 2. Response of nerve during passage of constant current. (*a*) "Cool" frog. Nerve at 17° C. after 3 hours' soaking in normal Ringer. Relative intensities (threshold 1): a_1, 5; a_2, 3; a_3, 1·5. (*b*) Nerve after soaking in Ca-free citrate Ringer at 17° C., showing an exceptional phenomenon, viz. slow, rhythmic, synchronized responses to current of 10 μA.

in normal Ringer's solution at room temperature (15–19° C.). These nerves usually gave marked multiple response to constant current, if of slightly more than rheobasic strength (Fig. 2). This was unexpected, since accommodation in frog's motor nerves at 15–19° C. is usually rather rapid: Solandt's [1936] average value was about 30 msec.: and the formula $T = \lambda \log_e I/I_0$, for I only slightly greater than I_0, gives T only

a small fraction of λ. Further experiments, however, showed that in these particular nerves the time factor λ was actually very great.

Certain phenomena regularly recorded in these experiments must be described in detail (Figs. 2 and 3). The repetitive nerve impulses diminished rather rapidly in size and frequency and soon became "asynchronous"; this occurred sometimes from the beginning so that the frequency could not then be measured. The interval between (initial) impulses was less with greater current strength, down to a value of about 2·5–3 msec. (frequencies estimated ranged between 80 and 200–400 impulses per sec.). The duration of response was greater with greater current intensity, and at twice the threshold was about 0·1–0·2 sec., varying in different preparations (see Table I below) and in different nerve elements.

Since perceptible electric responses of the nerve did not stop at any definite moment, but fell gradually to zero, the duration of maintained excitation could be estimated only approximately.

While Fig. 2*a* shows the regular behaviour, in rare cases (and these usually in Ca-free nerves and with rather high current intensity) prolonged apparently "synchronous" discharges of slow rhythm and maintained amplitude (see Fig. 2*b*) were observed. (It is doubtful, however, whether this exceptional phenomenon is due to the normal process of electric excitation or rather is associated with a latent state of spontaneous activity [see Biedermann, 1898].)

Direct comparison showed that repetitive electric response, to constant current applied to the nerve, lasted about the same time in muscle as in nerve.

The frequency of muscle response varied with intensity of stimulus. A greater $(V-U)$ would cause excitation earlier in the relative refractory period. At about 15° C., the highest frequencies observed were 150–200 per sec. The lengthening of interval between successive impulses was less distinct in muscle than in nerve response.

The repetitive action of the muscle fibres was, in most cases, "synchronous" for a considerable number of successive impulses (Fig. 3); the size of response decreased gradually (not as rapidly as in nerve, probably because simultaneous activity in different fibres was longer maintained). Eventually, only one or two "motor units" responded, outlasting the rest of the fibres by several impulses and stopping usually at a well-defined point. The duration, therefore, of multiple response in the motor nerve fibres can often be accurately determined.

The frequency of (initial) nerve impulses increased with frequency from 80 up to 200–400/sec., while a variation of muscle response from

80 up to only 150 (rarely 200 per sec.) was observed. This might suggest that, in some cases, during the initial stage of constant current flow, each second nerve impulse is blocked at the myoneural junction. The evidence, however, for this is not conclusive, because (i) the elements of highest

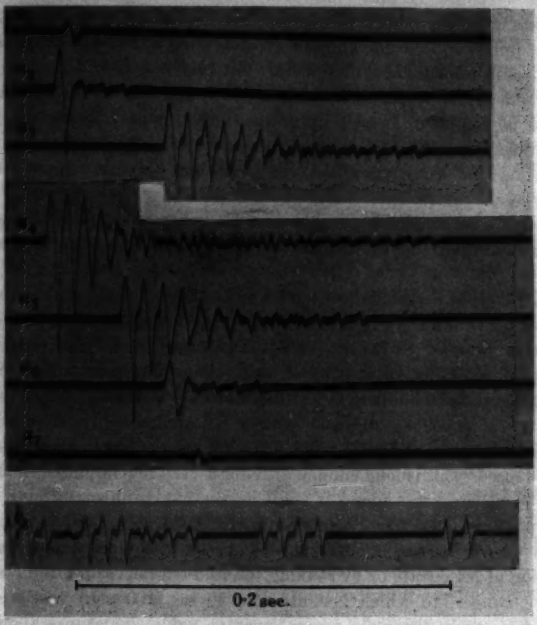

Fig. 3. Electric response of muscle during passage of constant current through nerve. (*a*) "Cool" frog, preparation at 14° C. after 4½ hours' soaking in normal Ringer. Relative strengths of constant current: a_1, 1·0; a_2, 1·33; a_3, 1·66; a_4, 2·33; a_5, 1·66; a_6, 1·33; a_7, 1·0. (*b*) Amplification 3·5 times higher than in (*a*). Record shows "intermittent failure" sometimes observed during decay of repetitive response. Twice threshold strength. Record started about 0·1 sec. after moment of closure.

frequency are not necessarily identical with the motor fibres and (ii) in estimating the frequency of nerve impulses an error due to asynchronous action of different elements cannot always be excluded.

It was considered whether the duration of the repetitive muscle response might possibly be reduced by a kind of Wedenski-block at the

nerve endings, which would prevent the use of the muscle response as index for the duration of maintained nerve excitation. This, however, could not be the case in these experiments: for (i) with greater current intensity (and greater frequency of nerve impulses), the duration of muscle response became considerably greater; and (ii) the muscle response declined finally in obvious steps (Fig. 3*a*); obviously whole motor units were ceasing, and not an increasing number of single muscle fibres (as in the case of a myoneural "fatigue").

The decaying response of the muscle showed sometimes intermittent groups of impulses, spaced by rather long "gaps". These are to be attributed, for the same reasons, to an intermittent failure in nerve excitation: for (i) synchronous intermittent response, apparently of whole motor units, was observed; and (ii) the gaps were either more marked, or occurred after a shorter time, when the intensity was low. Assuming a failure at the neuro-muscular junction, the contrary should be found.

(2) *Calcium nerves.* Injecting Ca into the animals, or soaking the isolated nerve for a few hours in a Ca-rich solution, quickens the accommodation process [Solandt, 1936]. The same procedure is found to reduce considerably, or even to abolish, the multiple response. Nerves soaked for about 6 hours in 8–10 times normal Ca responded to a current of 3 times threshold strength with 1–3 impulses only. The formula $T=\lambda \log_e I/I_0$, for $I/I_0=3$, gives $T=1{\cdot}1\lambda$, which for $\lambda=6$ msec. gives $T=6{\cdot}6$ msec.; this is long enough only for a very few impulses. A similar effect of calcium was found in crab nerves which normally give much more prolonged repetitive response [Katz, 1936].

(3) Ca-*free nerves.* Depriving the nerves of ionized Ca—either by injection of Na citrate or by soaking the isolated nerves for 2–5 hours in Ca-free Ringer, to which Na citrate (150 mg./100 c.c.) was added—increased λ to 10 times the normal average value, sometimes abolishing completely the ability to accommodate [Solandt, 1936]. Nerves which normally gave very little repetitive response (nerves of frogs acclimatized to room temperature, see Table I below) showed persistent repetitive response to constant current of little more than threshold strength. This again applied to the motor fibres as well as to the whole nerve.

After a few hours soaking in "Ca-free citrate Ringer", the nerves started firing off spontaneous discharges, in irregular and asynchronous sequence; they remained in continued "spontaneous" activity for hours, getting gradually into a state of "fatigue" and "refractoriness". It was sufficient to apply Ca-free citrate Ringer locally to an uninjured part of

the nerve in order to elicit spontaneous twitching. Removing the citrate Ringer and replacing it, after several times washing, by Ca-free Ringer (without citrate) did not diminish the continued activity. The twitching stopped immediately after cutting away the Ca-free part.

When citrate Ringer was applied to a normal rather rapidly accommodating nerve ("warm" frog), the state of spontaneous discharge was preceded by a remarkable lowering of threshold and a phase in which very prolonged response to constant current occurred. The threshold was lowered, by the removal of Ca-ions, to 1/8–1/10 of its original value (e.g. to 0·2 instead of 1·6 μA.), while soaking for a few hours in 10 times Ca raised the threshold about 3 times. The onset of spontaneous activity thus appears as the limiting result of (i) very slow accommodation and (ii) lowering of threshold, so that casual fluctuations of V make it greater than U_0.

Ca-free nerves, when stimulated in a state of prolonged spontaneous activity, exhibited distinct signs of "fatigue" and rapid lengthening of the refractory period at the point of stimulation [cf. Bugnard & Hill, 1935]. The response to repetitive stimulation at 200/sec. (condenser discharges of $RC = 0{\cdot}1$ msec., submaximal) was irregular: the first impulse being of greatest amplitude, followed by a considerable "refractory gap" and progressive failure in the different elements. In this state, constant current produces usually a few impulses only, rapidly disappearing and followed again by irregular spontaneous discharge.

(4) *Opening response.* The intensities required for break excitation were usually much higher than the threshold for a single closing response. More than two or three repetitive impulses, after break of constant current, were not observed in normal preparations (even at 7–10 times closing threshold strength). This is probably due to the well-known electrotonic changes of excitability at cathode and anode. The state of "normal accommodation" [Hill, 1936], in which the final steady value of $U-V$, during a continuously flowing polarizing current, is equal to U_0-V_0, independent of the sign of the local potential produced, is usually not present. The steady value of $(U-V)$ is generally increased at the anode, lowered at the cathode. (Hill [1935, Fig. 1]: the line OAA_1 meets the "accommodation curve" only at rather high intensities.)

Reversing the normal electrotonic effects would facilitate the occurrence of multiple opening impulses at relatively low intensities. Such states are well-known (e.g. Werigo's cathodal depression; see Cremer [1909], Biedermann [1898]; they result, for example, from application (*a*) of strong long-lasting current, (*b*) of low temperature [Eichler, 1933],

(*c*) of K-rich solution [Chweitzer, 1935; Bouman, 1936]; and (*d*) from fatigue and deterioration.

Isolated sciatics were kept at 4° C. and used at 2–5° C. After 7 hours no change of the normal state was observed, after 20 hours, however, the normal response to closing and opening of constant current was completely reversed. Repetitive opening response occurred at relatively low intensities (above about 2μA.), while the threshold for make response was much higher. (In this anomalous state, even at 10–20μA., only a small number of fibres responded to make of constant current.)

Fig. 4. Response of crab muscle during the passage of continuous current through the limb nerve. Time marks: 3 sec. Stimulus marks: rise shows "make", fall "break" of constant current. Intensities (volts) see record. + means "descending", − means "ascending" current. Note that with successive stimulations, the closing response fails, while the opening tetanus becomes enhanced.

The dependence of repetitive opening excitation on the electrotonic condition of the nerve appeared most clearly in the dissected crab nerve. Stimulation of limb nerves of *Carcinus maenas* with constant current produced at first prolonged closing tetani, while break of constant current was usually ineffective. With successive stimulations, however, the closing tetani became shorter and finally disappeared, while opening tetani appeared, which sometimes lasted considerably longer than the duration of previous current closure (see Fig. 4). The failure of make response could partly be reversed by introducing current pulses of opposite direction. These phenomena are much the same as Werigo's cathodal depression, viz. a reversal of the normal electrotonic excitability changes [see Cremer, 1909].

(5) *The relation between λ and T. Accommodation in "warm" and "cool" frogs.* The regular prolonged repetitive response in "cold frogs'" nerves, which had been soaked for many hours at room temperature, was surprising, although v. Frey [1883] had stated that the tetanic response

to constant current found in the isolated nerve-muscle preparations of cooled frogs may still be produced after a few hours at 15° C. There was an apparent discrepancy between Solandt's average value of λ (about 30 msec. at room temperature) and the duration of maintained response (about 0·2 sec. at 3 times threshold strength).

Determinations of λ in these preparations, however, settled this apparent disagreement. Nerves of "cool" frogs, used shortly after dissection, showed in some cases practically no accommodation at all. They gave persistent tetanic response even to threshold stimuli, the current intensity being independent of the time constant of current rise. During the first hour after dissection, λ was of the order of 300 msec., even after 6 hours at about 16° C., it was usually well above 100 msec. This agrees well with the observed duration of repetitive response to constant current.

Prolonged "injury discharges", after tying off the central end of the nerve, were often observed; the immediate twitch-like response during the ligature was followed, after a delay of a few seconds, by repetitive discharges continuing for a minute or more (in one case, it stopped only after 4 minutes, which approaches the similar phenomenon observed in crab motor nerve after amputation of the limb [Barnes, 1930]).

The time factor k of excitation [Hill, 1936] was determined in one experiment with single condenser discharge and a small muscle twitch as index: one hour after dissection, at 15° C., a k of 0·6 msec. was found (λ/k being about 500).

A clear relation between λ and T was found which will be discussed below. The greater λ, the longer was the duration of multiple response; this was the case when comparing different preparations as well as under different conditions in the same nerve. Nerves of cooled frogs soaked for a few hours in Ca-rich solution (8–10 times normal) showed a reduction of λ to less than 1/10 of its previous value, and at the same time abolition of repetitive response (Table I). Nerves of rapidly accommodating warm frogs soaked in Ca-free citrate Ringer showed a progressive lengthening of λ (increasing 10-fold in 4 hours) and a corresponding increase in T, until finally spontaneous activity prevailed. During prolonged experiments, λ and repetitive response were reduced both to the same degree. Further, nerves of any "cool" frogs which did not show marked multiple response, correspondingly had a small λ.

The great variation in respect of repetitive response [v. Frey, 1883; Fessard, 1935] which is found in different animals, presumably corresponds to the great variation of λ found by Solandt [1936]. Solandt's experiments suggest that these differences may be due to a variation in

the ionized serum Ca. The effect of acclimatizing frogs to different temperatures might possibly be due also to a change in the blood Ca-content and connected with the fact [de Boer, 1918; for further references see Morgenstern, 1933] that summer frogs have twice the blood Ca of winter frogs.

Discussion

It appears from these experiments that, in a general way, the theory of accommodation can be applied to the phenomena of multiple response. It remains to be seen how far the agreement is quantitative.

For the case of "normal" accommodation, i.e. neglecting electrotonic alterations of excitability, the change of $(U-V)$ following the "make" of a constant current through a nerve is given [Hill, 1936, equation (16)] by

$$U-V=(U_0-V_0)\left[1-\frac{I/I_0}{1-k/\lambda}\left(e^{-t/\lambda}-e^{-t/k}\right)\right].$$

If I is greater than the rheobase I_0, $(U-V)$ starts by being positive, then becomes negative, and finally becomes positive again. During the interval in which $(U-V)$ is negative repetitive excitation should occur. The end, therefore, of repetitive excitation is at time T given by putting $(U-V)=0$; hence

$$\left[1-\frac{I/I_0}{1-k/\lambda}\left(e^{-T/\lambda}-e^{-T/k}\right)\right]=0.$$

Unless accommodation is impossibly rapid T is great compared with k, so that $e^{-T/k}$ is effectively zero and we may write

$$e^{T/\lambda}=\frac{I/I_0}{1-k/\lambda}.$$

Since, in nerves which show repetitive response, k/λ is small, we may for simplicity neglect it, and finally obtain

$$T=\lambda \log_e I/I_0.$$

Thus for a current e times the rheobase (e being the base of Napierian logarithms) the interval T of repetitive response should be equal to λ, the time constant of accommodation. In Table I the interval T calculated from the above formula is compared with that actually observed in experiments on frog's nerve. Fig. 5 shows that the duration of repetitive response to a certain relative current strength agrees approximately with the calculated values (four preparations with different λ's). The agreement is better than was expected and probably gives an undue impression of accuracy. Actually, the figures of Table I and of Fig. 5 are affected by several possible errors: (i) average values were taken from several oscillo-

graph records, which showed often a large "scatter" of T; (ii) the determination of "rheobase" from the oscillograph records was not very exact; and (iii), in cases of very slow accommodation, λ could not be very

TABLE I. Accommodation and repetitive response. (Muscle response as index.)

		Multiple response		
λ sec.	I/I_0	Duration observed sec.	Duration calculated sec.	
0·135	3·0	0·2	0·15	4½ hours at 14° C. "Cool" frog
0·17	3·75	0·2	0·22*	6 hours at 14° C. "Cool" frog
0·18	3·0	0·22	0·2	5 hours at 14° C. "Cool" frog
0·3	2·0	0·2	0·21	1 hour at 15° C. "Cool" frog
0·02	4·0	0·0	0·028	After soaking in 9 times Ca
0·016	3·0	0·018	0·018†	"Warm" frog
0·013	3·5	0·012	0·016‡	"Warm" frog
0·03	3·5	0·036	0·038§	"Warm" frog
0·015	4·0	0·0	0·02	"Warm" frog
0·15	3·5	0·25	0·19‖	After soaking in Ca-free citrate Ringer

* At twice threshold: 0·107 sec. calculated, 0·04 sec. observed; at 10 times threshold: 0·39 sec. calculated, 0·4 sec. observed.

† Up to 2·3 times threshold, no "extra impulses".

‡ Up to 2·7 times threshold, no "extra impulses".

§ Up to 2·5 times threshold, no "extra impulses".

‖ Occasional spontaneous twitching was already manifest. Duration of repetitive response, therefore, could be estimated only roughly.

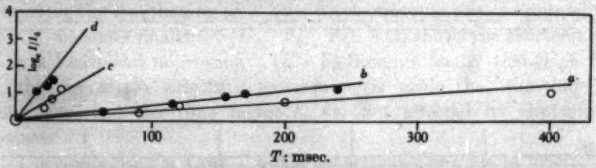

Fig. 5. Relation between multiple response and speed of accommodation. 4 preparations with different λ's. (a, $\lambda = 300$ msec.; b, $\lambda = 180$ msec.; c ("cool" frog, nerve with little repetitive response), $\lambda = 35$ msec.; d, $\lambda = 16$ msec. (up to 2·3 times threshold, no "extra impulse": not represented in the figure)). The plotted points show the observed duration T of repetitive response, in msec., against $\log_e I/I_0$. These values were obtained from oscillograph records (muscle response as index; average taken from two or three series of observations made before and after determination of λ). The lines give the slope $1/\lambda$.

accurately determined because the slope of the line relating threshold to rate of current rise was then very small. The exactness of the agreement, in some cases, may have well been due to favourable chance. In other cases, more distinct deviations were found (cf. Table I). T seemed often to be smaller than expected, if λ was small, it was sometimes even greater than expected if λ was great. The "scatter" of the observed values was

sometimes large, particularly in cases of small λ. In general, however, the agreement is satisfactory, considering the various disturbing factors involved.

The refractory period. The recovery of excitability following excitation, together with the value of $(V-U)$, presumably determines the frequency of repetitive response. The recovery of excitability is not

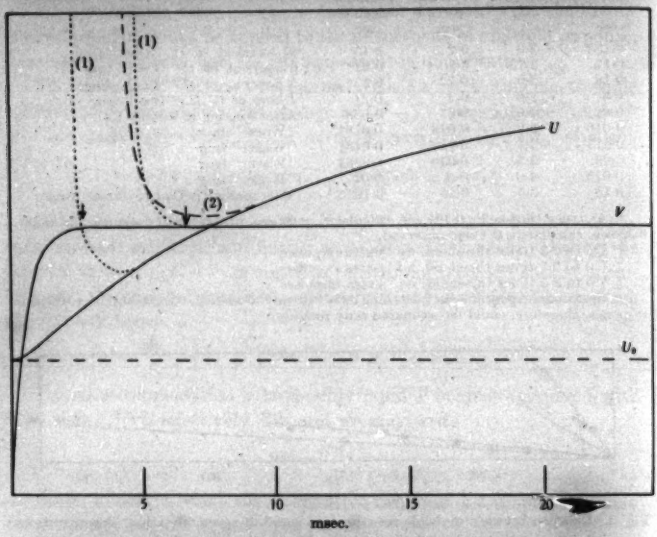

Fig. 6. Diagram illustrating influence of refractory period on multiple response. $k=0{\cdot}5$ msec., $\lambda=10$ msec. (1) absolute refractory period 2 msec., relative refractory period 5 msec. (2) absolute refractory period 4 msec., relative refractory period 9 msec. $I/I_0=2$. In case (1) two extra impulses (see arrows), in case (2) no extra impulse would occur during T. (T would be found experimentally as zero in case (2).)

necessarily the same as that following a single shock, since the refractory period is known [Bugnard & Hill, 1935] to be lengthened during "high-frequency", particularly one-way, stimulation. This lengthening of refractory period presumably occurs also in the repetitive response of nerve to constant current, which is clearly a limiting form of "high-frequency one-way" stimulation. A state of maintained local refractoriness might reduce, or even prevent, multiple response, even with slow

accommodation: this is probably the case in Ca-free nerves, after continued spontaneous activity.

When accommodation is quick, a variation in the length of the refractory period would produce a considerable change of the apparent value of T. If λ is short, e.g. 10 msec., and the relative refractory phase rather long, one may get, even at 3 times threshold intensity, no repetitive impulse at all, simply because the recovery period outlasts or is equal to T. This is illustrated in Fig. 6. In this figure the refractory period is shown as a variation in threshold after effective excitation. This is arbitrary, because what is actually known is only that, during the "recovery phase", stronger currents are required to make V equal to U. Whether this is due to a rise in "threshold" or a temporary drop in "local potential" is unknown.

If, however, λ is large (e.g. 100 msec.) there is time for many impulses to occur, and the observed value of T would not be considerably reduced by the failure of one or two final impulses.

Electrotonic changes of excitability. The extent to which the duration of repetitive response might be altered by a strong, enhancing, or depressing, effect of catelectronus, is illustrated in Fig. 7. Fig. 7 (1) shows the theoretical state: $(U-V)$ becomes finally equal to (U_0-V_0). Fig. 7 (2) illustrates the lengthening of T by a catelectrotonic rise of excitability: $(U-V)$ becomes finally 0·5 (U_0-V_0). Fig. 7 (3) shows the shortening of T by a "cathodal depression": $(U-V)$ becomes finally 1·66 (U_0-V_0). In this figure the arbitrary assumption is made that the shape of the curve, and the time factor λ, are not affected by electrotonic changes.

The comparison of λ and T would not seriously be affected by electrotonus, because both were determined under similar electrotonic conditions. A variation, however, of refractory period and electrotonic changes during successive stimulation might conceivably account for the sometimes considerable "scatter" of the observed values.

Repetitive response to a single strong shock. A peculiar deduction may be drawn from the theory, namely that a strong shock, even of very short duration, may produce an extra response. With a strong enough shock V is raised a long way above U and then falls: if it has not fallen below U by the end of the refractory period a second excitation must occur. This is illustrated in Fig. 8 for the case of a crab's nerve with $k=2$ msec., a recovery period lasting 4 msec., and an instantaneous shock of 8 times threshold. A condenser discharge of duration $RC=0·5$ msec. and of three times threshold should be sufficient to produce two

impulses. The longer the duration of the discharge the less the
of the current need be for a second impulse.

In frog's nerve, k is so short compared with the refractory pe
excitation by very short shocks could not in practice lead to m
one response: with longer shocks, however, particularly i
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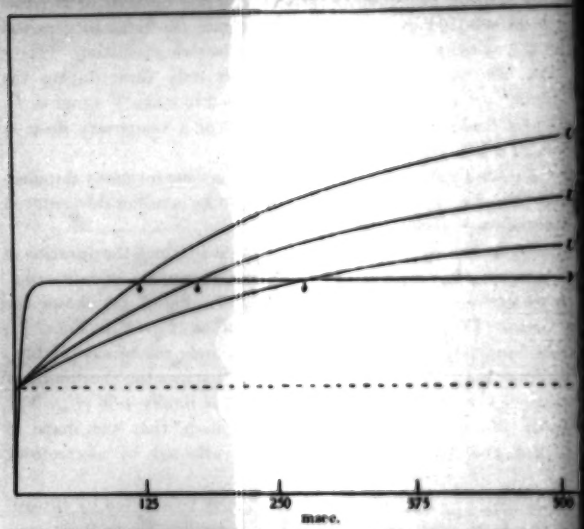

Fig. 7. Diagram illustrating threshold changes during passage of consta
$k = 5$ msec., $\lambda = 250$ msec. (In order to make the rise of V visible, a k has be
about 10 times greater than the actual value.) The curves illustrate th
influence of electrotonic excitability changes. (1) theoretical state; (2) ris
ability by catelectrotonus (Pflüger effect); (3) lowering of excitability by
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The response to linearly progressive currents. Hoffmann [1
shown that the response of frog's nerves to linearly increasing
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In frog's nerve, k is so short compared with the refractory period that excitation by very short shocks could not in practice lead to more than one response: with longer shocks, however, particularly in slowly accommodating nerve, repetitive response should be easy to obtain.

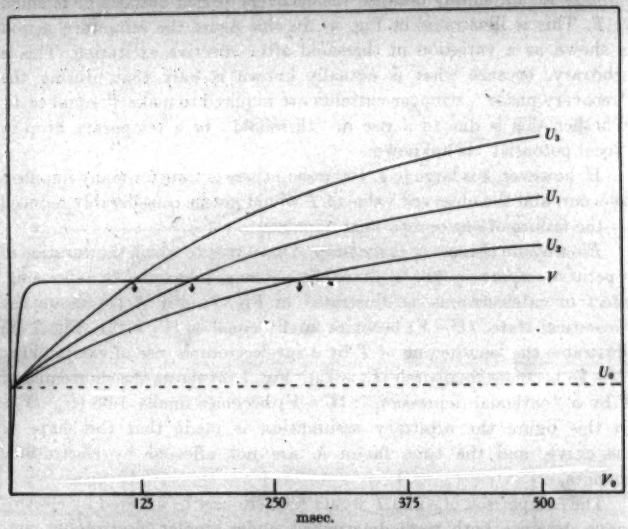

Fig. 7. Diagram illustrating threshold changes during passage of constant current. $k=5$ msec., $\lambda=250$ msec. (In order to make the rise of V visible, a k has been chosen, about 10 times greater than the actual value.) The curves illustrate the possible influence of electrotonic excitability changes. (1) theoretical state; (2) rise of excitability by catelectrotonus (Pflüger effect); (3) lowering of excitability by catelectrotonus (Werigo phenomenon).

The response to linearly progressive currents. Hoffmann [1910] has shown that the response of frog's nerves to linearly increasing current (muscle action potential as index) always consists of a train of impulses lasting as long as the current rises. This is in agreement with Hill's scheme [1936, p. 312, Fig. 5]: with this particular form of stimulus, only two possibilities exist: (i) the rate of current increase in smaller than the "minimal gradient", V then never reaches U and excitation does not

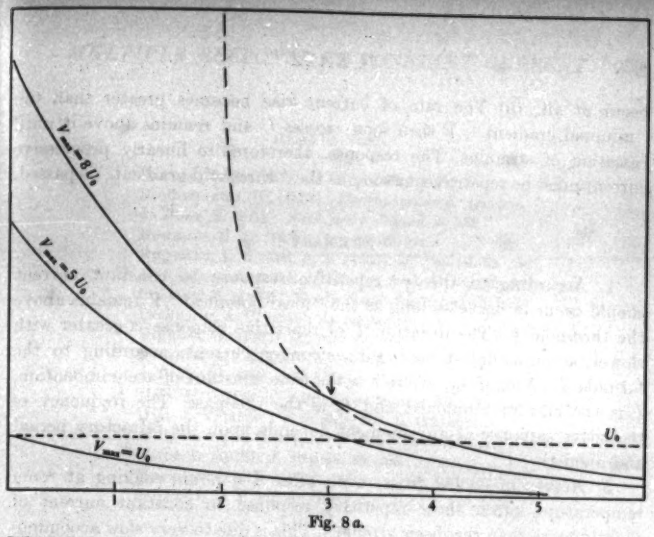

Fig. 8 *a*.

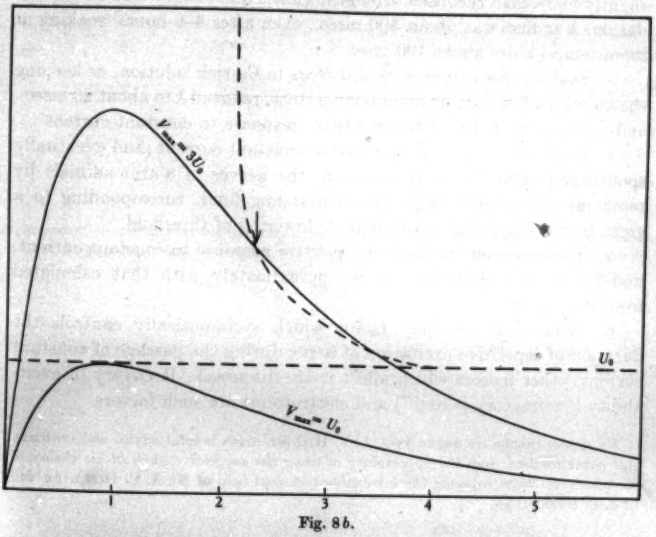

Fig. 8 *b*.

Fig. 8. Diagram illustrating after-effect of short condenser discharges. (*a*) "instantaneous" discharge, $RC = 1$ μsec. $k = 2$ msec. λ about 1 sec. Absolute refractory period 2 msec., relative refractory period 4 msec. At 8 times threshold strength one extra response charge may occur (arrow). (*b*) condenser discharge, $RC = 0{\cdot}5$ msec. k, λ, and refractory period as in (*a*). At 3 times threshold strength, one extra response may occur (arrow). Abscissa: msec.

occur at all. (ii) The rate of current rise becomes greater than the "minimal gradient", V then soon crosses U and remains above it until cessation of stimulus. The response, therefore, to linearly progressive current must be repetitive, as soon as the "threshold gradient" is passed.

Summary

1. According to theory, repetitive response to constant current should occur in nerve so long as the "local potential" V remains above the threshold U. The duration T of repetitive response is greater with slower accommodation and with stronger current, according to the formula $T = \lambda \log_e I/I_0$, where λ is the time constant of accommodation, I is the current employed and I_0 is the rheobase. The frequency of repetitive response at any moment depends upon the refractory period and upon $(V-U)$.

2. Nerves of cooled frogs, even after 4–6 hours soaking at room temperature, often show repetitive response to constant current of slightly more than rheobasic strength. This is due to very slow accommodation: λ at first was about 300 msec., even after 4–6 hours' soaking at room temperature above 100 msec.

3. Soaking the nerves of cooled frogs in Ca-rich solution, or keeping the animals a few days at room temperature, reduced λ to about 20 msec., and reduced or abolished the repetitive response to constant current.

4. Prolonged repetitive response to constant current (and eventually spontaneous activity) is produced in the nerves of warm animals by removing the Ca-ions from the surrounding fluid, corresponding to a great increase in λ and a considerable lowering of threshold.

5. The observed duration of repetitive response to constant current, under various conditions, agrees approximately with that calculated from the theory.

6. While λ is the only factor which systematically controls the duration of repetitive excitation of nerve during the passage of constant current, other factors which affect it are discussed. Recovery of excitability ("refractory period") and electrotonus are such factors.

My sincere thanks are due to Prof. A. V. Hill for much helpful advice and criticism. This paper resulted from the opportunity of using the suggestive ideas of his theory of excitation, and from enjoying the invaluable technical help of Mr A. C. Downing and Mr J. L. Parkinson.

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DILATATION OF VEINS IN RESPONSE TO TAPPING IN MAN AND IN CERTAIN OTHER MAMMALS

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(*Received* 17 *June* 1936)

We recently noted the great and rapid dilatation of the abdominal vena cava of the rabbit and the cat, which occurs when this vessel is tapped with the forefinger [Franklin & McLachlin, 1936 *a*, *b*]. Goltz [1864] found that the frog's mesenteric vessels dilated in response to this form of stimulation, and other veins have from time to time given similar results, but so far as we know there has been no systematic examination of a large number of veins in different species. One of our present objects is to provide some such comprehensive survey, and to attempt an explanation of the findings. The other is to emphasize the clinical possibilities of this method of stimulation, for we think that its use might perhaps have saved a certain patient's life. This patient had suffered a severe hæmorrhage, and in consequence her cutaneous veins were so strongly constricted that a necessary intravenous injection could not be made. Had we heard of this case in time, we should have suggested that tapping might relieve the spasm, for in our earlier experiments with rabbits we had an almost parallel case, in which injection was only made possible by such means.

Method and results

We examined the response to tapping of a number of veins in two guinea-pigs, seven hutch rabbits, two cats, one dog, and a number of human beings. The cats were anæsthetized with chloroform and ether, and anæsthesia then continued with ether only; all the other animals were anæsthetized with ether. In the guinea-pig we tested the ilio-renal vena cava, the portal, superior and inferior mesenteric veins; in the rabbit the ilio-renal vena cava, the short gastric veins and various larger branches of the portal system, the right testicular, left renal, azygos and intercostal veins; in the cat the ilio-renal and reno-diaphragmatic vena cava,

the portal, superior and inferior mesenteric, splenic, left renal, femoral and external jugular veins; in the dog the ilio-renal vena cava, portal vein, inferior and superior mesenteric branches, short gastric, splenic, femoral and external jugular veins.

Dilatation produced by tapping was almost invariably confined to increase in cross-section, but the ilio-renal vena cava in one cat and in one rabbit showed in addition a certain increase in length and consequent tortuosity. An increase in length was also produced by compression of the vessel, and it was so marked in the cat that the vein actually became partially kinked. The increase in cross-section was presumably due to relaxation of "circular" muscle, in length to relaxation of "longitudinal muscle".

In every case in which quantitative measurements of the changes in calibre were possible, we calculated the increases in cross-section produced. When these increases were tabulated, we found that no general conclusions could be drawn from them, for the degree of dilatation varied from 10 to nearly 500 p.c., and there was no correspondence between the findings for corresponding veins; in other words, the initial calibre of the veins was dependent on a number of uncontrollable factors. We thought, therefore, that we might more profitably compare the maximal dilatations produced by tapping in the same vein in animals of the same species. Our previous results had indicated as a probability that the calibre after tapping would be greater with increase in body weight. In the present series the two guinea-pigs weighed respectively 300 and 350 g.; the calibres of the venae cavae after tapping were respectively 2·3 and 2·4 mm., though the corresponding increases in initial cross-section were 83 and 376 p.c. The second cat weighed 2·1 times as much as the first, the cross-section of its ilio-renal vena cava after tapping was 2·18 times that in the first cat, and the corresponding ratios for the splenic and portal veins were 1·9 and 1·8. The percentage increases in cross-section, on the other hand, were 31 and 21 for the venae cavae, 140 and 35 for the portal veins, and 40 and 89 for the splenic veins. In order to confirm the suggestion that the cross-section after tapping was directly related to the body weight, we examined the reactions of the abdominal venae cavae of five rabbits of different weights. Four of these were lean, the fifth was very fat; we considered that we were justified in subtracting the weight of the abdominal fat from the body weight of this fifth rabbit, for this fat was very avascular. On dividing the cross-sections of the tapped venae cavae in sq. mm. by the weights of the animals in kg., we obtained the figures 18·4, 16·2, 19·1, 18·7, and 16·8; the weights ranged

from 1250 to 2150 g. When we take into account the difficulty of being quite certain that a maximal stimulation had been given, and the difficulty of measuring rapid fluctuations in calibre, these results may be regarded as indicating that the cross-sections of the veins after adequate tapping are directly proportional to the body weight of the animals in a particular species.

The ilio-renal vena cava responded to tapping most readily in the guinea-pig and the rabbit, and least in the dog; the cat was intermediate. We think that this variability may perhaps be correlated with the normal habits of the different species, the more so as the wild rabbits in our earlier experiments approximated to the cat type rather than to the hutch rabbit type. From the dog to the guinea-pig and hutch rabbit the relative weight of the splanchnic viscera increases, and the ability for sustained muscular exercise decreases. The rabbit's vena cava thus tends to constrict more than that of the dog in the resting condition, for relatively more blood will go through the shorter splanchnic circuit in the less active animal. There was some variability in the readiness of the response to tapping in the five rabbits; in one of them maximal dilatation occurred with a single tap, in others several taps were required.

The renal vein dilated readily in the rabbit and the cat, but was completely irresponsive in the one dog; it was perhaps already maximally dilated in this last animal. In the dog the vein which dilated with most ease was the splenic, and this observation is perhaps to be correlated with the high degree of spleen reservoir function in this species.

The azygos vein in the rabbit showed no response to tapping until the interference produced by blood waves from the right auricle was removed; it then dilated appreciably.

The short gastric veins dilated on tapping only so far distal as their exits from the serous coat of the stomach. The same was the case with the short intestinal veins. The femoral veins, which are firmly supported by connective tissue, were irresponsive. All the other veins, which we have listed above, except the dog's renal vein, responded to tapping by dilatation. It appears to us, therefore, that the dilator response is confined to veins which possess some active circular muscle and which are not surrounded by strong supporting structures, such as the muscular coats of the viscera or relatively dense connective tissue.

In human beings we made no actual measurements, as the skin obscures somewhat one's view of the subcutaneous veins, and consideration for the patients negatived any prolonged procedures in the case of internal veins exposed during operations. The changes in calibre

were in any case sufficiently obvious without measurement. The subcutaneous veins, which can be examined in the intact person and which respond to tapping by dilatation, include certain of those on the dorsum of the hand and foot, and those in the arm and leg; the last-named are more difficult to see than the others, because of the perivascular subcutaneous tissue. The superficial inferior epigastric vein, exposed during laparotomy, also dilated on tapping. Deeper veins, which it was found possible to test, were all responsive. They included mesenteric veins from the ileum and transverse colon, the appendicular vein, the venous plexus in front of the bladder, and the pampiniform plexus. So far as they go, therefore, the findings in normal human beings correspond well with those in lower animals; the case of the abdominal vena cava must, however, await a direct observation, for its structure in human beings is different from that in pronograde mammals [Franklin, 1932]. Varicose veins, which were examined, showed some dilatation on tapping with the patients in the supine position; they dilated still more, however, under hydrostatic pressure when the patients stood upright, so the neuro-muscular apparatus could not have been very efficient in these veins.

Summary

1. The response of veins to tapping has been examined in the guinea-pig, rabbit, cat, dog, and man.

2. It is widely distributed, but appears to be confined to veins which are not firmly supported by surrounding structures.

3. Independent of the initial calibre of a susceptible vessel, tapping appears to increase its cross-section to a maximum, which is directly proportional in any particular species to the body weight of the individual.

4. When venous spasm prevents intravenous injection into an experimental animal or a human patient, it is worth while to tap the vein in the expectation of dilating it.

Since this account was written, Dr Una Fielding and Dr Keith Richardson, of University College, London, have found that the internal jugular veins of Macaque monkeys also respond to tapping by dilatation, the effect being more marked in the younger animals.

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THE REACTIONS OF THE ABDOMINAL VENA CAVA

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(*Received* 17 *June* 1936)

In previous papers we gave an account of the reactions of the ilio-renal vena cava [Franklin & McLachlin, 1936 *a*, *b*]. We did not consider it necessary to study the innervation of the thoracic inferior vena cava in the common laboratory mammals, as it is for practical purposes devoid of smooth muscle in these animals [Franklin, 1931, 1932, 1933 *a*], and its changes in length and cross-section are governed by other factors [Franklin, 1933 *b*, 1934; Franklin & Janker, 1934, 1936]. To complete our studies of the inferior vena cava, therefore, we examined the reactions of its reno-diaphragmatic portion.

Method and results

The main work was done upon rabbits, anæsthetized with ether, but we also tested the effect of tapping in one cat. The general procedure was similar in all cases; the thoracic part of the right sympathetic chain and the portion of vein under consideration were exposed by resecting the last six ribs on the right side, the animal being given artificial respiration as required. The sympathetic chain was divided at the level of the sixth rib and prepared for distal faradic stimulation. The strength and duration of stimulation were similar to those which we employed for our earlier experiments on the ilio-renal vena cava.

In every case the effect was similar, so it will suffice to give details of one. The initial calibre of the vein was 7·4 mm. and the nerve was stimulated for 30 sec. At 10 sec., 1, 1½, 2, 3, 4, 5, and 7 min. from the cessation of the stimulus the calibre was respectively 6·7, 5·0, 5·8, 5·2, 5·6, 5·9, 6·5, and 6·5 mm. The diminution in cross-section at the end of 1 min. was therefore 54 p.c. The second diminution in calibre, at the end of 2 min., we attributed to secretion of adrenaline, and in our opinion the

same factor was responsible for the persistent slight constriction of the vein at the end of the period of observation. We attempted to settle the question by removing the adrenals, but the trauma to the glands during their removal was severe and the calibre of the vein dropped to 4·6 mm., presumably through a further, more copious discharge of adrenaline. It is known that the secretion constricts this portion of the vena cava, though to a less extent than it does the more distal portion [Franklin, 1933 *a*]. In another rabbit the primary constriction, at 1 min. from the cessation of the stimulus, was less than the secondary one at 200 sec.

The reno-diaphragmatic vena cava in the resting rabbit and cat is not subject to the same degree of natural constriction as is the ilio-renal vena cava. In view of our hypothesis about the meaning of the dilatation produced by tapping [Franklin & McLachlin, 1936 *b*], we thought that this relative lack of constriction might be due to the large and continuous inflows of blood into this portion of vein. We therefore clamped off the right renal vein in one rabbit and measured the resulting diminution in cross-section of the vena cava; it was 16 p.c. With a vein so dilated from the beginning as the reno-diaphragmatic vena cava, one would not expect any large further dilatation on tapping; we have, nevertheless, found appreciable increases of the order of 12 p.c. in cross-section.

Summary

1. Distal faradic stimulation of the right thoracic sympathetic chain produces a constriction of the reno-diaphragmatic portion of the rabbit's inferior vena cava.

2. Removal of the inflow from one renal vein produces a diminution in cross-section of the vena cava.

3. Tapping the vein causes it to dilate.

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STREAM-LINES IN THE ABDOMINAL VENA CAVA IN THE LATE STAGES OF PREGNANCY

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The genital veins in the female cat and in the doe rabbit contain highly saturated blood, and the inflows from them into the vena cava produce red stream-lines within the larger vessel [Franklin & McLachlin, 1936]. Sir Joseph Barcroft suggested that, as the uterine blood becomes more desaturated in the later stages of pregnancy [Barcroft & Rothschild, 1932], the stream-lines in the vena cava should become less apparent at that time.

We have since been able to confirm this suggestion through the courtesy of Dr R. G. R. West, who allowed us to examine a chloralosed cat, upon which he was operating. The rarity of opportunities for inspecting animals at a sufficiently late stage of pregnancy is our excuse for reporting a single case instead of a series. The right horn of the uterus in the animal in question was empty, but its diameter—*c.* 1·5 cm.—suggested recent parturition. In the left horn was a practically full-time fœtus; this was rapidly removed, through a cut on the side opposite the large vessels, with a negligible loss of blood, and the wound was immediately closed by a continuous suture. In the vena cava there was a thin red stream-line from the left uterine vein, a wide one from the right uterine vein and also one from the right ovarian vein. The streams showed up more clearly when artificial respiration was given, as the breathing under the chloralose was very shallow. Some time after removal of the fœtus, the left horn had contracted down to approximately the same diameter as the right horn. The fœtus measured 12·5 cm. from vertex to buttock, and its claws and hair were well developed; if anything, it seemed larger than ordinary kittens are at birth. We presume, therefore, that parturition was imminent and that we could not have found a more suitable case in which to test Sir Joseph Barcroft's suggestion.

SUMMARY

The increasing desaturation of the blood within the uterine veins, during the later stages of pregnancy, reduces the degree of redness of the stream-lines, which are produced within the vena cava by the inflows from these veins.

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THE ACETYLCHOLINE METABOLISM OF A SYMPATHETIC GANGLION

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Although the distribution of cholinergic fibres has been widely studied of recent years, little is yet known of the processes underlying the discharge of acetylcholine by nerve impulses. Such evidence as is at present available suggests that the acetylcholine transmitting the effect of a nerve impulse is not formed by the arrival of the impulse, but is mobilized from a preformed store. Experiments of Witanowski [1925] and Engelhart [1930] suggested that vagus stimulation might cause an increase up to 500 p.c. in the amount of acetylcholine extractable from the heart. Vartiainen [1934] was unable to confirm these findings, and the very short refractory period of the vagal mechanism found by Brown & Eccles [1934] is difficult to reconcile with an immediate synthesis. Chang & Gaddum [1933] have also found that stimulation of the chorda-lingual nerve does not significantly affect the amount of acetylcholine extractable from the submaxillary gland. Even, however, if it is true that acetylcholine is liberated by the nerve impulse and not synthesized, resynthesis must occur at some time, if the tissue is to retain its acetylcholine, and the problem of acetylcholine formation, as Vartiainen has pointed out, is merely shifted to a later stage.

The perfused superior cervical ganglion of the cat provides an organ especially suited to the study of this problem, since a considerable volume of quantitative data concerning its acetylcholine metabolism is already available. The tissue is of fairly constant weight, in the region of 10 mg., consists almost entirely of nerve cells and nerve endings, and yields a very high acetylcholine equivalent on extraction, which seldom varies more than a few p.c. between the right and left ganglion in the same animal, so that a convenient control is available.

[1] Rockefeller Foundation Fellow.

Feldberg & Vartiainen [1934] have already made careful measurements of the amounts of acetylcholine liberated from a ganglion, and have been able to determine the quantity liberated by each preganglionic stimulus during short periods of stimulation. A simple calculation showed that, if this rate of output were maintained during a more prolonged stimulation, the store of acetylcholine present in the ganglion would, in the absence of new formation, rapidly become exhausted. This has been tested experimentally in the experiments here recorded. The acetylcholine output of a perfused ganglion during prolonged preganglionic stimulation has been followed in detail, and the quantity of acetylcholine finally extractable from the ganglion has been compared with that from the non-stimulated ganglion of the other side. From the data obtained we have been able to establish that acetylcholine synthesis does, in fact, take place during a prolonged stimulation, and some of the factors concerned in this synthesis have been studied. In the course of these experiments a condition of partial paralysis of the ganglion resulting from preganglionic stimulation has been observed, and an analysis of the effect is included in this paper.

A preliminary account of these experiments has already appeared in this *Journal* [Brown & Feldberg, 1936*c*, *d*].

Methods

Perfusion of the superior cervical ganglion of the cat was carried out in the manner described by Feldberg & Gaddum [1934] and Feldberg and Vartiainen [1934]. It was particularly important in these experiments to ensure that the fluid perfusing the ganglion was free from any trace of blood. This was achieved by very careful preparation, especially of the postganglionic trunk, in which fine anastomizing arterial twigs may easily escape ligature, and by additional ligation of the common carotid artery and the vagus nerve on the cardiac side of the perfusion cannula. The perfusion fluid was oxygenated Locke's solution to which was added eserine, when necessary, to give a concentration of $1 : 5 \times 10^5$. In these conditions, the venous outflow from the ganglion amounted to between 2 and 5 c.c. in 10 min. in different experiments, but in any one experiment it could be kept constant by occasional adjustment of the perfusion pressure.

The preganglionic fibres of the cervical sympathetic were stimulated with break induction shocks, timed by a Lewis contact breaker, delivered through fluid electrodes of the Collison [1933] pattern, filled with the defibrinated blood of the animal under experiment.

The retraction of the nictitating membrane was recorded with an isotonic lever with a magnification of 11 and a tension of some 12 g. on the membrane.

The acetylcholine content of the venous effluent was estimated on the dorsal body-wall muscle of the leech sensitized with eserine and, in some experiments, a second assay was done on the cat's blood-pressure. So well did these agree, that in later experiments entire reliance was placed on the leech test. In some experiments, choline was added to the perfusion fluid. This substance, if in sufficient concentration, may cause contraction of the leech. We therefore ascertained that the leech preparation did not react to solutions containing choline in excess of the concentration likely to appear in the venous effluent.

Ganglion extracts

The ganglia were extracted with 10 p.c. trichloracetic acid in the manner previously described [Brown & Feldberg, 1936*a*] and their acetylcholine content assayed on the leech, the frog's *rectus abdominis*, or the cat's blood-pressure. In those experiments in which the extractable acetylcholine from the normal ganglion was compared with that from the ganglion after prolonged perfusion and stimulation, the stimulated ganglion was excised immediately after stopping stimulation. The "resting" ganglion was then subjected to some 10 min. perfusion with fluid of the same composition as that used for the other side, and was then excised. To avoid errors due to spontaneous discharge from cut nerves, both cervical sympathetics were cut as soon as possible in the preliminary dissections.

Choline estimations

We have estimated the choline content of ganglion extracts and of ganglion perfusates. The procedure with ganglion extracts was as follows. The method for acetylcholine extraction was followed as far as the completion of the removal of the trichloracetic acid with ether, but care was taken to allow extraction to proceed for a fixed time of 45 min. This time was chosen arbitrarily, so that, if any breakdown of lecithin or other precursors of choline occurred, this might be approximately constant. After removal of acid, the extract was transferred with alcohol to a hard glass tube and dried over-night in an oven at 110° C. Sufficient acetylchloride completely to cover the dried residue was added, the tube was sealed and immersed for 1 hour in a boiling water bath. The tube was cooled, opened and the acetylchloride removed in an evacuated desiccator containing calcium chloride and caustic potash. The residue was dissolved

in saline at *p*H 4, so as to give a solution containing the equivalent per c.c. of 5 mg. of the wet weight of the ganglion. The acetylcholine equivalent of the acetylated extract was estimated on the frog's *rectus abdominis* and on the rabbit's isolated jejunum. Closely concordant results were obtained if no more than 0·2 c.c. of the extract was added as a dose to the intestine bath (15 c.c.). Even with such doses, allowance has to be made for an unexplained depressant effect of the extract on the response to the succeeding dose of the standard solution of acetylcholine, and this standard dose must be repeated. If larger amounts of extract are used, the intestine is so much depressed, that it is not possible to obtain an estimate comparable with that obtained on the frog's rectus.

The venous effluent, containing large amounts of salt in proportion to the choline present, was evaporated to dryness under low pressure. The dried residue was then taken up with dry alcohol, filtered, and the filtrate transferred to the acetylation tubes. After acetylation, the residue was dissolved in acid saline to give a concentration of 1 c.c. ≡ 4 c.c. of the original perfusate. The rabbit's intestine proved to be the most satisfactory test object, although the strength of the solutions was at the limit of the sensitivity of the intestine to acetylcholine.

Results

Effects of prolonged preganglionic stimulation

Contraction of nictitating membrane. The contraction of the nictitating membrane, recorded during prolonged maximal or nearly maximal stimulation of the preganglionic fibres, with the ganglion perfused with Locke's solution containing eserine, has the following form. With the start of stimulation, the curve of contraction rises steeply, but the ascent is interrupted after some 5 sec. by a partial relaxation, which is maximal after 2–3 min. From this point recovery takes place to a level higher than the original crest. After this second maximum has been attained, the contraction declines very slowly as long as the stimulation is continued. A particularly good example of such a contraction curve is shown in Fig. 1, and a less pronounced effect in Fig. 3.

If, during such a prolonged stimulation, the output of acetylcholine from the ganglion is measured, it is found that in the first 5 min. the output is at a high level, the concentration being as high as 1 in 10^7. From this value, the production falls off rapidly along an approximately exponential curve, until, 20–60 min. after the beginning of the stimulation, the concentration of acetylcholine in the venous effluent is only

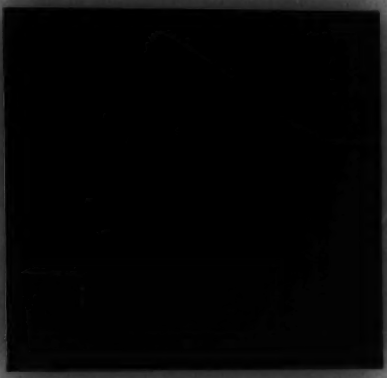

Fig. 1. Contraction of nictitating membrane of cat. Ganglion perfused with Locke's solution containing eserine $1 : 5 \times 10^5$. At signal, stimulation of cervical sympathetic with maximal break shocks at 11 per sec.

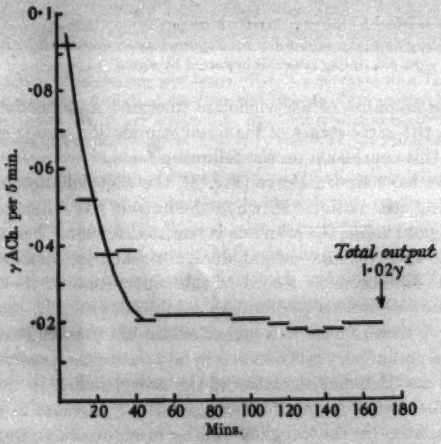

Fig. 2. Output of acetylcholine from perfused ganglion. Maximal preganglionic stimulation at 17 per sec.

1 in 2×10^8 (Fig. 2). Comparing the contraction of the nictitating membrane with the corresponding output of acetylcholine, it is seen that the slow recovery of contraction occurs during the rapid fall of the output of acetylcholine, and that the maximum contraction may be reached when the acetylcholine output has only 1/5 of its original value.

This lack of correlation between contraction of the nictitating membrane and the output of acetylcholine led us to attempt further analysis of the effect. We found that the form of the earlier part of the contraction curve, with the relaxation from the preliminary crest, is not due to changing responsiveness of the nictitating membrane, but to changes in the ganglion. It is due to depression of the response of the ganglion cells

Fig. 3. Contraction of nictitating membrane. Ganglion perfused with Locke's solution containing eserine $1 : 5 \times 10^8$. Maximal preganglionic stimulation at 15 per sec. interrupted by two intervals of 3 min. Figures below record show output of acetylcholine in γ per c.c. during intervals indicated by signal.

by the large amounts of acetylcholine liberated and persisting in the ganglion, in the early stages of maximal stimulation under eserine. We have based this conclusion on the following facts.

(*a*) As we have shown above (Fig. 2), the acetylcholine output falls rapidly during stimulation. If, when the output has fallen to less than 1/3 of its original value, the stimulus is remitted for some 3 min. and then renewed, the acetylcholine output does not reach its former high level (Fig. 3). The simultaneous record of the contraction of the nictitating membrane now shows no preliminary inhibition (Fig. 3), and the contraction curve rises at once to a higher maximum than the original.

(*b*) If the preliminary relaxation is in fact due to the paralysing action of excess of acetylcholine, increase of the acetylcholine in the ganglion by any means should cause a further inhibition. Increase in the acetylcholine circulating in the ganglion can be brought about by increasing a just submaximal stimulus until it is maximal, or by actual injection of acetylcholine into the fluid perfusing the ganglion. These effects are

illustrated in Fig. 4. At *B*, stimulation of the preganglionic sympathetic was started at a frequency of 10 per sec. and the secondary coil at 8 cm. At the signal *C* =6, the secondary coil was advanced 2 cm. and produced a precipitate relaxation which continued with little change when the stimulation was returned to its previous lower strength. At the next signal the stimulus was again increased during the recovery from "inhibition". There is again a prompt relaxation. At *C*, 0·4γ acetylcholine was injected, and this also caused relaxation. The same dose injected at *A*—previous to stimulation—had caused contraction. After

Fig. 4. Contraction of nictitating membrane. Ganglion perfused with Locke's solution containing eserine $1:5\times10^6$. *A*, injection into cannula of 0·4γ acetylcholine. *B*, preganglionic stimulation, 10 per sec.; coil 8. *C*=6, increase in strength of stimulation. *C*, *D*, injection of 0·4γ acetylcholine. Between *C* and *D*, 44 min. continued stimulation

C, stimulation was continued for 44 min., until the acetylcholine output had fallen. Repetition of the increase in stimulating current, or renewed injection of acetylcholine at *D*, both now caused further contraction.

(c) If the destruction of acetylcholine in the ganglion is not prevented by addition of eserine to the perfusion fluid, none of these "inhibitory" effects occurs, the contraction of the nictitating membrane, during continuous preganglionic stimulation, rising along a smooth curve to a maximum from which it slowly and steadily declines (Fig. 5).

The inhibitory phenomenon appears to occur only in the perfused ganglion. We have attempted to produce it in the ganglion with natural circulation and have given doses of eserine up to 7 mg. per kg. This amount of eserine causes severe muscular twitching which interferes with recording. To avoid this, we have used a decerebrate preparation with the

carotid arteries left open and have administered a few mg. of curarine. Curarine, as we have shown previously [Brown & Feldberg, 1936*b*], paralyses the ganglion, and this we have also observed in the ganglion with natural circulation. The paralysis of the ganglion, however, soon passes off, leaving the skeletal muscles still insensitive to eserine. Under these conditions, in spite of the large dose of eserine given, stimulation of preganglionic fibres yields a smooth contraction of the nictitating membrane without any sign of the "inhibitory" phases.

In Fig. 5 an interesting subsidiary phenomenon is recorded. The signal line above the time record shows the rate of falling of the drops from the venous cannula. Before stimulation the rate was one drop in 8 sec., and

Fig. 5. Contraction of nictitating membrane. Perfusion with Locke's solution without eserine. *A*, at signal, maximal preganglionic stimulation at 12 per sec.; signal line above time record shows drops of venous outflow. *B*, after 37 min. continued stimulation.

after the start of stimulation the rate fell to 1 drop in 10 sec. This slight, but evident vaso-constriction is not seen in the ganglion perfused with a solution containing eserine, in which stimulation may be followed by some vaso-dilatation.

Synthesis of acetylcholine during prolonged stimulation

In Fig. 2 is shown graphically the output of acetylcholine during a stimulation lasting 3 hours. The ordinates show the amount of acetylcholine estimated as present in each sample collected over 5 min., the abscissæ represent time in minutes. As already pointed out, the output falls steeply along an approximately exponential curve for some 20–60 min., until a value of 1/5th or even 1/10th of the original output is reached. At this point, the steep exponential fall ceases and the output remains steady, with only a slight decline, as long as stimulation is continued. If the amounts determined in each sample are added, it is found that the total quantity of acetylcholine liberated from the ganglion

in the particular experiment illustrated is 1·02γ. This is greatly in excess of the quantity of acetylcholine obtainable from an unstimulated ganglion by extraction.

In a previous paper [Brown & Feldberg, 1936*b*] we have given some figures of the acetylcholine obtainable by extraction from ganglia. These figures were obtained from ganglia excised without interruption of their blood supply, and in order adequately to control the present experiments, we have made extracts from ganglia after prolonged perfusion with Locke's solution alone and with Locke's solution containing eserine. We have found (Table I) that perfusion, with or without eserine, does not affect the acetylcholine content of the ganglion, beyond the limits of normal variation. We find, moreover, that prolonged stimulation does not apparently bring about a significant diminution in the extractable acetylcholine present (Table II).

TABLE I. Acetylcholine content of ganglia. Control perfusion experiments in which the preganglionic fibres were not stimulated

	Perfusion fluid Locke solution +	Duration of perfusion min.	Acetylcholine extracted from ganglion	
			Absolute amount	γ per g.
1.	Eserine	120	0·15γ	18
	—	—	0·13γ	14
2.	Eserine	60	0·33γ	24
	—	60	0·20γ	20
3.	—	120	0·31γ	19
	Choline	120	0·40γ	24

TABLE II. Effect of stimulation on acetylcholine content of ganglia. Perfusion fluid – Locke's solution + eserine. Choline added in exp. 4.

	Duration of stimulation min.	Total output acetylcholine	Acetylcholine extracted from ganglion	
			Absolute amount	γ per g.
1.	113	0·42γ	0·17γ	13·0
	—	—	0·27γ	19·0
2.	160	0·57γ	0·28γ	24·0
	—	—	0·18γ	17·0
3.	229	1·31γ	0·20γ	15·5
	—	—	0·28γ	22·5
4.	135	0·82γ	0·40γ	21·0
	—	—	0·25γ	15·0

In the experiment illustrated in Fig. 2 the ganglion of the unstimulated side yielded on extraction 0·28γ acetylcholine, or 22·5γ per g. The stimulated ganglion, which had already discharged 1·02γ acetylcholine, yielded

0·2γ acetylcholine (15·5γ per g.). The total amount of acetylcholine yielded by the stimulated ganglion is thus 1·22γ, as compared with 0·28γ from the unstimulated side. Clearly therefore nearly 1γ acetylcholine, corresponding to four times the amount present in the normal ganglion has been synthesized during this stimulation. In some experiments in which, in the later stages, the output was augmented by various means, the total output amounted to 1·3γ, or about five times the quantity extractable.

If the time relations of the output are studied in greater detail, it is found that an amount of acetylcholine is liberated in the first 20–40 min. of stimulation equal to or even greater than the extractable acetylcholine. In many experiments we found that the amount liberated, up to the time at which the curve of output changes from the exponential fall to the "steady output", is of the same order as the amount extractable. For instance, in four experiments, the quantities of acetycholine liberated up to the end of the exponential fall were 0·26, 0·27, 0·44 and 0·31γ. These figures are surprisingly near in value to the average amount of acetylcholine which we have found in ganglia in the fourteen extractions shown in Tables I and II, namely, 0·25γ.

Augmentation of output of acetylcholine

(*a*) *Blood.* In one experiment, in which isolation of the minute vessels of the postganglionic bundle was not complete, we found that a temporary lowering of the perfusion pressure caused the appearance of blood in the venous effluent. This was associated with a sudden increase in the amount of acetylcholine liberated, and a simultaneous increase in the contraction of the nictitating membrane. In this particular instance, 80 min. after the beginning of stimulation, the acetylcholine output had fallen to 0·018γ in 10 min., and the contraction of the nictitating membrane to 58 p.c. of its original value. After the admission of blood, the acetylcholine rose to 0·025γ in 10 min., and the contraction of the membrane to 68 p.c. of the original height. This effect we have been able to repeat with regularity by the actual injection of minute amounts, not only of whole blood, but also of plasma, into the perfusion cannula. Such injections cause a decrease in the rate of venous outflow, which must be compensated by an increase in perfusion pressure. In one experiment, after 1 hour of stimulation, a 10 min. sample had a concentration of acetylcholine of $1:2\times10^8$ (0·0095γ), and the contraction of the nictitating membrane had fallen to 45 p.c. of its original value. After injection of 0·1 c.c. of a 10 p.c. dilution of whole blood in Locke's solution, the next

sample of 1·7 c.c. contained acetylcholine $1:1{\cdot}2\times10^{8}$ (0·014γ) and the nictitating membrane contraction increased to 60 p.c. of the original. After a further 30 min. stimulation, a second injection of 0·5 c.c. 10 p.c. blood raised the concentration of acetylcholine in the perfusate from $1:2\times10^{8}$ to $1:6\times10^{7}$; the venous outflow, however, decreased about 50 p.c. Nevertheless, the actual amounts of acetylcholine liberated in 10 min., before and after the blood, were 0·005γ and 0·01γ respectively. A similar augmentation of output, as noted above, was produced with equal regularity by the injection into the cannula of plasma or serum, a fact which indicates that the effects are not attributable merely to an improved oxygenation of the perfused tissues.

These results suggest that the beneficial effects of blood or plasma are due to the replenishing in the ganglion of some essential material for the acetylcholine synthesis, which, during prolonged stimulation, the saline perfusion removes or fails to replenish. If this is true, then no such diminution as we observed in the perfused ganglion should be detectable in the acetylcholine output of a ganglion with its natural blood supply, beyond that resulting from fatigue of the preganglionic fibres. This seemed the more likely, in that the contraction of the nictitating membrane supplied by a ganglion with natural circulation was maintained during prolonged stimulation with little diminution. We have obtained evidence of the maintenance of the acetylcholine output under such conditions, from one experiment in which the venous blood was collected from a ganglion before stimulation, at the beginning of stimulation, after 1 hour and after 2 hours' stimulation of the preganglionic fibres. The ganglion was prepared as for perfusion, so that the blood collected flowed only from the tissues usually perfused. The blood was rendered incoagulable by successive injections of chlorazol-fast pink, and the cat (3 kg.) received before each collection of blood 1 mg. atropine and 0·9 mg. eserine intravenously. The venous outflow was very slow, but remained constant, and the acetylcholine concentration showed no falling off, a concentration of $1:4\times10^{7}$ appearing in the first sample after stimulation was begun, and a concentration of $1:3\times10^{7}$ in the sample collected at the end of 2 hours' stimulation. Carotid arterial blood, collected as a contrast immediately after the last sample, showed no detectable acetylcholine.

(*b*) *Choline.* We have attempted to determine which constituent of injected blood or plasma could be responsible for the augmentation of acetylcholine output. The first substance tested and, incidentally, the only substance found effective, was choline. An experiment in

which the action of choline is particularly well illustrated is shown in Fig. 6.

After 65 min. preganglionic stimulation the output in each 5 min. sample had fallen from 0·05γ to 0·009γ, and the contraction of the nictitating membrane had declined to 56 p.c. of the original value. The

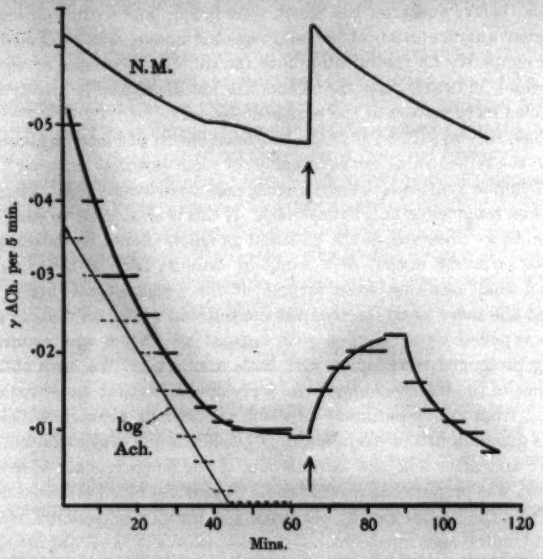

Fig. 6. Lower curve: output of acetylcholine from perfused ganglion; preganglionic stimulation at 10 per sec. Upper curve: height of contraction of nictitating membrane; at arrow, injection into cannula of 10γ choline chloride in 2 cc.

injection of 2 c.c. of a solution of choline chloride containing 5γ per c.c. caused a gradual increase in the acetylcholine output up to 0·022γ in the 5 min. sample, and an increase of the contraction of the membrane to a little beyond its first maximum. Later in the same experiment, a similar though smaller augmentation followed the injection of 1γ of choline chloride in 1 c.c.

In demonstrating this action of choline, care must be taken that the doses used are well below the threshold of stimulation, since choline

itself has, in sufficient dose, a stimulating action upon the ganglion cells [Feldberg & Vartiainen, 1934]. This stimulating action is seen when the substance is given in concentrations of the order of 10γ in 0·1 c.c. In these experiments doses of choline up to 10γ were used, but these were diluted in 1–5 c.c., and this concentration is well below the threshold of stimulation.

Although choline in some experiments had this dramatic effect, yet its action was irregular, and the augmenting effect was only obtained in five of the ten experiments in which it was used. We have been unable to find any correlation between time of administration, level of acetylcholine output, duration of stimulation and the effectiveness or otherwise of injected choline. In one experiment perfusion was carried out from the beginning with Locke's solution containing the usual concentration of eserine and, in addition, choline chloride in a concentration of $1:5\times10^5$. There was a suggestion of a longer delay than usual in the fall of output to a steady level, but the difference was of doubtful significance.

(*c*) *Other substances.* The apparently haphazard action of choline led us to suspect that some other substance, necessary for the re-synthesis, might be wanting in those experiments in which choline failed. Accordingly, sodium acetate, sodium aceto-acetate and a highly active preparation of cholinesterase were used, either alone or in combination with one another, or with choline. None of these experiments gave a clear positive result.

Choline metabolism of the ganglion

The remarkable activity of choline, which we have observed in certain experiments, suggested that there might exist in the ganglion a metabolism of choline, related to preganglionic stimulation, comparable to that of acetylcholine. We have therefore determined the output of choline in the venous effluent and the amounts of choline extractable from ganglia.

The venous effluent from the ganglion perfused with normal Locke's solution, without preganglionic stimulation, usually showed minute but detectable quantities of choline. The concentration varied between $1:30\times10^6$ and $1:80\times10^6$. As we have pointed out in a previous paper [Brown & Feldberg, 1936*a*], some acetylcholine may be detected in the venous effluent early in the perfusion of a ganglion in the absence of stimulation, but the concentration is rarely greater than $1:100\times10^6$, and even this amount is absent if the preganglionic fibres are divided some hours before the perfusion is started. Stimulation of the preganglionic fibres causes an increase in the total choline present in the venous outflow.

In samples collected during the first 30 min. of stimulation, the choline was usually present in a concentration of $1:16\times10^6$ and the absolute amount collected was 0·3—0·37γ. In later samples the concentration fell to $1:36\times10^6$, and in the second 30 min. of stimulation the absolute amount collected was about 0·16γ choline. In these experiments the choline assayed in the perfusate included the acetylcholine present, and a comparison of the figures with those for the acetylcholine output of the stimulated ganglion shows that the choline in the venous outflow can all be accounted for by the acetylcholine liberated by stimulation. In a few experiments we have estimated separately the total choline content and the acetylcholine in the venous effluent during stimulation, and find agreement between the two amounts as close as could be expected under the conditions of the experiment.

In Table III are shown the results of the determinations of the total free choline content of ganglia. The amount present varies between 243 and 520γ per g. of tissue, or roughly ten times the amount of acetylcholine

Table III. Choline content of ganglia

	Perfusion fluid Locke solution +	Duration of perfusion min.	Duration of stimulation min.	Choline content of ganglion: Absolute amount	Choline content of ganglion: γ per g.
1.	—	—	—	4·4γ	312
	—	—	—	4·0γ	243
2.	—	160	—	3·5γ	267
	—	—	—	3·8γ	308
3.	Eserine	180	130	4·4γ	440
	Control	—	—	3·6γ	315
4.	—	180	120	4·3γ	320
	Control	—	—	4·2γ	300
5.	—	133	65	4·1γ	350
	Control	—	—	4·2γ	350
6.	Eserine	170	120	4·9γ	512
	Control	—	—	5·4γ	520

present. Perfusion alone, with or without eserine, and stimulation of the preganglionic nerves, cause no significant alterations in the choline content. In one respect, however, the behaviour of the choline differs from that of acetylcholine. We have shown previously [Brown & Feldberg, 1936*a*] that degeneration of the preganglionic fibres of a ganglion is accompanied by a ten-fold reduction in the acetylcholine extractable. We have tested the effect of denervation on the choline content of the ganglion and find it to be unaffected. Thus, the cervical sympathetic on one side was divided

aseptically in a cat, and degeneration was allowed to proceed for 27 days. The ganglia were then excised and extracted, and each yielded 300γ per g. of choline.

Discussion

In these experiments we have shown that the acetylcholine liberated in a ganglion by preganglionic stimulation can, if protected by eserine, rise to such a concentration that it produces a partial paralysis of the ganglion cells. The paralysing effect of artificially administered acetylcholine on ganglion cells was described by Feldberg & Vartiainen [1934], who showed that acetylcholine injected into the fluid perfusing a ganglion has its stimulant action cut short by a secondary paralysing action. On the assumption that acetylcholine acts as the synaptic transmitter in the ganglion, we are now confronted with the difficulty that the same substance liberated at synapses can simultaneously excite and depress. We know that, with artificially injected acetylcholine, a small increase above the concentration required to give a maximal response of the ganglion cells, as judged by the contraction of the nictitating membrane, produces a smaller effect, and a long-lasting depression of the response of the ganglion to preganglionic stimulation, or to further injections of acetylcholine. It appears, therefore, that the ganglion cell has two thresholds, one for stimulation and one for depression, which are only separated by a narrow difference in concentration of acetylcholine. When high concentrations of acetylcholine are allowed to persist in contact with the ganglion cells, the concentration in relation to some of the cells may rise above the threshold of paralysis, and, these cells ceasing to transmit, produce the depression in the contraction curve of the nictitating membrane.

Recently, Brown, Dale & Feldberg [1936] have shown that acetylcholine injected into the arterial tree of a skeletal muscle, though acting as an immediate stimulus, may be followed by a severe depression of the response of the muscle to nerve stimulation, if its rapid destruction is prevented by eserine. They have suggested that the locus of the paralysing effect is the general body of the muscle fibre, rather than the end plate at which excitation occurs. One of their reasons for this suggestion is that in the perfused muscle, when œdema has delayed removal by the circulation, even doses of acetylcholine too small to excite may yet produce considerable depression of the motor nerve twitch. It is not inconceivable that a similar phenomenon may exist in the ganglion, viz. that acetylcholine, liberated at the synapses on the dendritic processes of the cell, may there only excite, whereas excess diffusing into

contact with the perikaryon may there depress the response of the neurone to stimuli incident upon its dendrites. It is perhaps significant, in this connexion, that we have been unable to obtain the paralysing effects of stimulation in the ganglion with its circulation intact, however great the amounts of eserine given. The fact that the acetylcholine liberated in a ganglion is closely restricted under normal conditions to the point of its excitatory action is shown by the vaso-constriction which follows stimulation in the ganglion perfused without eserine. This is apparently due to stimulation of postganglionic vaso-constrictor fibres in the cervical trunk; in the presence of eserine it is overpowered by the vaso-dilator action of acetylcholine diffusing from the site of its liberation. It may be noted that Eccles's [1935] suggestion, that one of the normal functions of acetylcholine liberated in a ganglion may be to cause vaso-dilatation, is not in accordance with this experimental evidence.

This secondary, paralytic effect of acetylcholine, if caused by eserine to persist in the ganglion, obviously complicates the picture, and makes it difficult to predict whether, in a particular case, eserine will cause a potentiated response to preganglionic stimulation, as Feldberg & Vartiainen observed under particular conditions, or whether such an effect will be obscured by depression of the responsiveness of the cells. Altogether, however, the evidence which we have presented involves no difficulty for the theory that acetylcholine normally transmits the excitation at the synapse, but supports it with further instances of close parallellism between the effects of nerve stimulation and the actions of acetylcholine.

Our experiments show that even the ganglion perfused with Locke's solution is capable, during prolonged stimulation, of synthesizing relatively large amounts of acetylcholine. As shown in the experiment illustrated in Fig. 2, a ganglion with an acetylcholine content originally of the order of 0·2γ is capable of discharging into the perfusate approximately five times that amount. We have no reason to suppose that this synthesis is affected by the presence of eserine, since the total choline output of a stimulated ganglion, perfused without eserine, corresponds closely, in amount and time relations, with the output of acetylcholine under eserine. It would appear, therefore, either that the enzyme responsible for this acetylcholine synthesis is not cholinesterase, or that eserine inhibits the hydrolytic action of this esterase, but leaves it capable of the opposite, synthetic action.

With regard to the manner in which a nerve impulse causes the appearance of acetylcholine on reaching the ending of the nerve fibre,

two possibilities have been the subject of much discussion. One is the new synthesis of acetylcholine from its constituents. Earlier experiments on the frog's heart were supposed to give support to this view, by showing that acetylcholine accumulated in the muscle during stimulation of the vagus nerve. Later experiments have not confirmed this suggested accumulation, and our experiments on the ganglion are, at least, decisive on this point. Stimulation of the preganglionic fibres of the perfused ganglion, with or without eserine, causes neither increase nor decrease in the amount of acetylcholine which can be extracted from the tissue. On the other hand, it causes the output of a quantity several times as great as that which can be extracted at any stage, so that new formation of acetylcholine is apparently occurring, whatever may be the relation of this process to the arrival of impulses at the nerve ending. These facts are compatible with the view that each impulse synthesizes the acetylcholine required to transmit its effect across the synapse, and that the quantity extractable from the ganglion has no direct relation to this process. It is not easy, however, to imagine such a synthesis taking place in some fraction of the short period of the synaptic delay, or within the refractory period. From this point of view, it is much easier to imagine that the acetylcholine, which appears with the arrival of the impulse at the preganglionic ending, is liberated from a pre-existing complex, in which it is stored in an inactive form and protected from destruction. On this supposition the early outburst of acetylcholine, during a prolonged stimulation of the perfused ganglion, would be explained by a depletion of this depot, subsequently maintained at a relatively low level by synthesis meeting the diminished output. The figures for the acetylcholine extractable from the ganglion, however, give no greater support to such a conception, than to that of direct synthesis by each impulse; they afford no evidence of an accumulation which the earlier stages of the stimulation deplete. It is possible that the depletion may occur in a reserve of choline, but again, estimation of the extractable choline showed that no change was produced by stimulation. It must be pointed out, however, that although the choline is greatly in excess of the amount necessary for acetylcholine synthesis, we have no reason to suppose that the choline obtained by extraction is available for synthesis; in fact, the finding that denervation leaves the choline content unaltered, points to its being of cellular origin and not, like the acetylcholine, dependent on the integrity of the preganglionic terminals. It is, of course, conceivable that an exhaustion of the choline store of the preganglionic terminals might occur, which would be masked by the large amounts present in the

ganglion as a whole. In any case, if a "depot" theory is adopted, it can only be made to fit the facts by assuming that the quantity of acetylcholine obtainable from a ganglion by artificial extraction does not represent what is available at the preganglionic terminals for liberation. A possible variant of the depot theory would suppose that acetylcholine is synthesized at the terminals, not at the arrival of the impulse, to act as an immediate stimulus, but during the ensuing refractory period. In this case it must be held in inactive form until a further impulse releases it. Such a conception, however, would involve at least as great a difficulty, from the brevity of the time available, as that of immediate synthesis in active form.

We cannot, on the evidence at present available, come to a final decision between the two possibilities. Most of the evidence would be compatible with either; but it must be admitted that, in spite of the difficulties of transmission time and refractory period, the theory of immediate synthesis by each impulse affords an easier and more direct interpretation of the immediate increase seen in output of acetylcholine when blood or plasma is admitted to the perfusion or, in many experiments, when choline is added to it. Neither theory, on the facts already known, can assign a suitable role to the acetylcholine or the choline obtainable from the ganglion by extraction.

Summary

1. The effects of prolonged preganglionic stimulation on the contraction of the nictitating membrane and on the output of acetylcholine from the perfused superior cervical ganglion of the cat has been studied.

2. During such stimulation, the acetycholine output starts at a high level and falls rapidly during the first 20–60 min., after which it continues at a steady low level with only a slight decline.

3. In the perfused ganglion, in the presence of eserine, the concentration of acetylcholine produced in the initial stages of stimulation is sufficiently high to cause partial paralysis of the ganglion cells.

4. During a stimulation of the preganglionic fibres lasting some hours, synthesis of acetylcholine appears to take place, since the total amount liberated may be several times that obtainable from an unstimulated ganglion by extraction. This synthesis is not affected by eserine.

5. Prolonged stimulation does not significantly alter the amount either of acetylcholine or of choline extractable from a ganglion.

We wish to express our thanks to Sir Henry Dale for the stimulating interest he has taken in this work.

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REFLEX SLOWING OF RESPIRATION ACCOMPANYING CHANGES IN THE INTRAPULMONARY PRESSURE

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(*Received* 22 *July* 1936)

In a paper published in 1932 we described acceleration of the breathing as the constant and normal response to collapse of the lungs. Creed & Hertz [1933] brought forward evidence which they believed showed that, while this was true with slight degrees of deflation, with higher deflation pressures a well-marked slowing replaces the acceleration.

We hope to be able to show that this reflex slowing only occurs when the negative pressure considerably exceeds that required to produce complete collapse of the lungs, and cannot, therefore, be due to impulses arising from the alveoli, but must originate as the effect of either pressure or distortion affecting the air passages (trachea or bronchi) or their surroundings.

The pressure required to produce complete deflation of the lungs either by increased external or decreased intrapulmonary pressure, varies in different animals: in rabbits being [illegible]2 to 15 mm. Hg, in dogs between 20 and 40.

If Creed and Hertz's observations be examined, the animal used being the rabbit, it is evident that the suction pressures which were accompanied by slowing of the breathing were much above the limit beyond which the lungs can be no further collapsed, the lungs containing only the residual air. In their Fig. 2 (p. 92 *l.c.*) two examples are shown of the slowing reflex, in one of which the pressure was −26 mm. Hg, in the other −35.

It is our intention in this communication to show:

1. That the reflex slowing described by Creed & Hertz is not related to a change in volume of the lungs.

2. That the reflex arises from inhibitory stimuli due to the distortion of the larger air passages or their intrathoracic surroundings, and that

it has probably the same origin as the slowing of the respiratory rhythm accompanying closure of the air exit in the expiratory phase of breathing.

3. That pressure changes within the air passages or lungs which occur as the result of closure of the tracheal outlet or suction from the trachea do not give rise to any reflex slowing of the rhythm when unaccompanied by any change in the volume of the thorax or distortion of the air passages.

Section 1. *The conditions under which reflex slowing of the respiration occurs with deflation*

Fig. 1 illustrates the effect in the rabbit of the gradual increase of intrapulmonary negative pressure to a point in excess of that required to produce complete collapse of the lungs.

Method. Recording was from the diaphragmatic slip. To obtain rigid fixation of the diaphragmatic ends of the slips the following modification of the usual method has been employed. A stout needle with a flat head 5 mm. in diameter, protected by a rubber washer of the same size, was passed from below through the diaphragmatic end of each slip and the thoracic wall about 1 cm. on either side of the sternum. The ends of the needles were then drawn up through the chest wall and firmly clamped at the end of a $\frac{3}{8}$ in. brass rod in contact with the thoracic wall and sternum, the rod being fixed in any desired position in a boss-head on a vertical upright attached to the operating table.

Using this method of fixation we find that no movements of the thorax affect the record, and that the sudden rise in the base line accompanying deflation of the lungs which might readily be interpreted as a tonic inspiratory response, is absent.

The rise in the base line shown in Fig. 1, Graphs 1 and 3, is not an effect of tonic contraction, but merely the fusion of successive accelerated cycles prolonged by the deeper breathing.

Deflation of the lungs was produced by a rapid lowering of the pressure in a 10 litre bottle attached to the tracheal cannula (through inspiratory and expiratory valves); the volume of air in the enclosed space into and out of which the animal breathed being sufficiently large, as compared to the tidal air volume, to prevent breathing being hampered. The intrapulmonary pressure was recorded by a Hg manometer connected to the tracheal outlet. Graph 2 in the figure shows the normal acceleration in the breathing accompanying deflation. The intrapulmonary pressure was not reduced below -14 mm. Hg, the rate of respiration rose from 48 to

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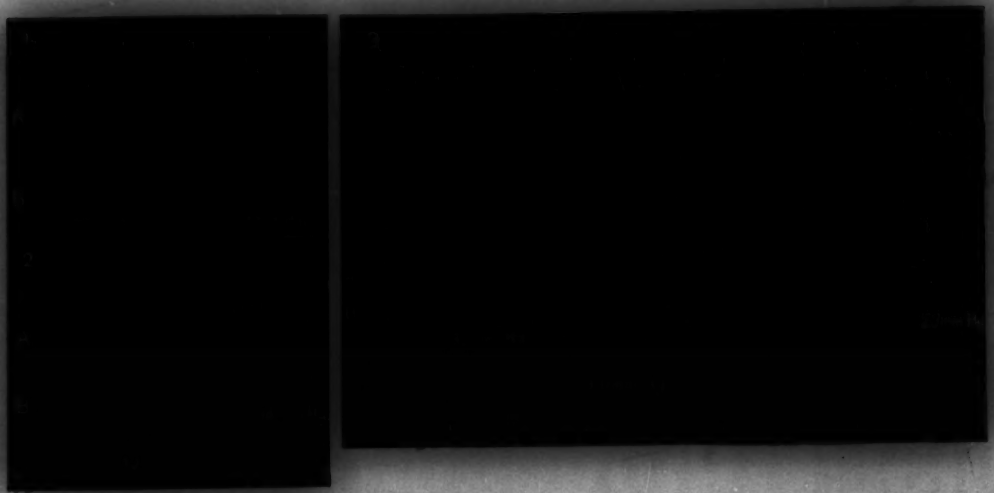

Fig. 1. Pressure conditions causing reflex slowing of breathing. Rabbit, 1630 g. Medinal, 0·8 g. Medinal at 8.50 a.m. Slips prepared at 10 a.m. A, Diaphragmatic slip record. B, Hg manometer.

Graph 1. Initial rate of rhythm, 47 per min.; accelerated, 82; slow, 44; negative pressure in mm. Hg at which slowing began, 26 mm. Hg; min. pressure, 38 mm.

Graph 2. Initial rate, 46 per min.; accelerated, 82 per min.; min. pressure, 14 mm. Hg.

Graph 3. Gradual increase followed by gradual decrease of negative intrapulmonary pressure. Initial rate, 44 per min.; (*a*) initial acceleration, 66 per min., (*b*) slow rhythm, 36 per min., (*c*) terminal acceleration, 66 per min.; pressure at which slowing began, 30 mm. Hg; pressure at which slowing gave place to acceleration, 20 mm. Hg.

Insp., up; exp., down. Read L. to R. Time, 10 sec.

82 per min. It is to be noted that an intrapulmonary pressure of −14 is just below that required to produce complete collapse of the lungs in the rabbit. In Graph 1 the pressure was gradually lowered to −38 mm.: it will be observed that when the pressure had reached −26 the initial acceleration gave place to a well-marked slowing of the rhythm, which continued until the pressure was restored to normal. In Graph 3 the pressure was reduced fairly rapidly to −60 mm., maintained at that level for about 12 sec., and then gradually restored to the normal.

It will be seen that the initial acceleration gave place at a pressure of −30 mm. Hg to a marked and increasing slowing of the rhythm accompanied by a great augmentation in the amplitude of the diaphragmatic slip contractions, and that during the release of the pressure the accelerated rhythm returned when the pressure had reached −20 mm.

It is clear that the negative pressures at which slowing replaced acceleration or vice versa in the experiment illustrated in Fig. 1 are considerably greater than would be needed to cause complete deflation of the lungs.

In an experiment conducted in a similar manner in a dog (record by stethograph) the intratracheal pressure was gradually reduced to −80 mm. Hg and then gradually increased. At a pressure of −40 mm. the accelerated rhythm of 90 per min. changed to a rate of 39 per min., and so continued until during release the pressure had reached −35 mm.

When the lungs are fully collapsed, by opening the pleura or by the method used in these experiments, their volume cannot be further reduced. It follows therefore that the effect of any excess of pressure must fall solely on the air passages, and that any reflex response arising under such circumstances must originate in the walls of the trachea and bronchi or their immediate intrathoracic surroundings. It may therefore be justly assumed that inhibitory stimuli are set up by the excitation of the vagus nerve endings in these structures and that, when deflation has reached its maximum limit, the onset of reflex slowing depends on the point at which the inhibitory influence preponderates over the augmentor influence arising in the collapsed lungs.

With deflation of the lungs by external pressure a reflex slowing, relative to the accelerated rate due to deflation, but not equal to the initial rate of breathing, is occasionally observed in the rabbit but has not been seen in the dog: this may be accounted for by the more rigid thoracic walls in the latter animal. It is also clear that the condition established in the walls of the air passages and the mediastinal and other thoracic viscera are essentially different in the two procedures, deflation

by suction and by external pressure, quite apart from the fact that in the latter the intrapulmonary pressure undergoes no change.

With suction from the trachea, combined with the inspiratory efforts to expand the thorax unbalanced by the resistance of extrathoracic pressure, the walls of the air passages and the surrounding tissues must be put into a state of tension, increased by each inspiration, such as cannot occur if the deflating pressure be external. Whatever distortion occurs must, in the latter case, be due solely to the diminished volume of the thorax produced by the compressing force. This difference has been pointed out by Adrian [1933, p. 345, par. 3] when discussing the origin of the electrical changes in the vagus.

SECTION 2. *Reflex effect of changes of pressure within the trachea alone*

When discussing [1933] the possible influence of the respiratory changes in the aperture of the glottis on the form of the respiratory cycle,

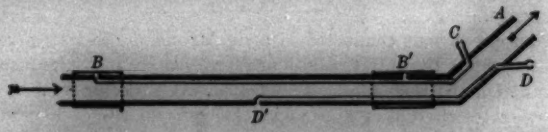

Fig. 2. Cannula for inflating or deflating a segment of the trachea. *A*, metal tube, 8 mm. internal diameter. *B*, *B'*, rubber sheaths surrounding *A*. *C*, fine tube running down inside *A* through which *B* and *B'* can be inflated; distance between *B* and *B'* = 10 cm. *D*, fine tube opening on outside of *A* at *D'* through which air can be injected or withdrawn from the space between *B* and *B'*. Arrow: air-way through cannula from lungs to outlet.

we drew attention to the fact that pressure changes in the upper air passages (glottis and above) caused a reflex slowing of the breathing (Fig. 9 of that paper) which was ascribed to the excitation of the termination of the superior laryngeal nerves by the changes in shape of the glottis produced by the pressure.

To determine whether, in a similar manner, changes of pressure in the trachea could cause a reflex slowing of respiration the following method has been employed. The cannula, shown in Fig. 2, was devised for the purpose.

The purpose of the cannula is to enable distension or collapse of the wall of the trachea to be effected without interfering with the breathing or with the nerve supply of the mucosa or other structures of the tracheal wall.

Method. The cannula is introduced through an opening in the trachea, cut transversely, 3 cm. below the larynx. It is about 12 cm. long and it is pushed in until the lower distensible sheath, *B*, is just above the bifurcation, and the upper, *B'*, just below the tracheal opening. *B* and *B'* are then inflated by forcing air in through the tube *C*. A section of the trachea between *B* and *B'* is thus shut off, breathing continuing through *A*, the lumen of which is sufficiently large for the purpose in a 6 kg. dog.

Distension or collapse of the tracheal wall is brought about by producing, through the tube *D*, a positive or negative air pressure within the space surrounding the tube *A*. Recording was by bellows recorder connected through valves and a reservoir to *A* or by a stethograph.

Fig. 3. Reflex effects of expansion and collapse of a segment of the tracheal wall.

Graphs I and II, dog 7 kg.; III and IV, dog 7·25 kg. In both cases 0·6 c.c. 5 p.c. morphine followed by 1·5 g. medinal intravenously. Upper line: record of respiration by a stethograph on lower end of sternum. Time record, 10 sec. Hg manometer record in III and IV showing negative intrapulmonary pressure. Graph I. *E*, reflex effect of expansion of tracheal segment, and *C*, collapse of segment. Graph II. Deflation of the lungs between the arrow marks; at *C* the tracheal wall was collapsed.

Graph III. Collapse of tracheal wall between the arrow marks at *C*.

Graph IV. Deflation of the lungs by negative pressure during the period shown on the manometer record, and collapse of the tracheal wall between the arrow marks at *C*. Inspiration up in all records.

The reflex effect of distension of the tracheal segment is seen in Fig. 3, Graph I, at *E*, of collapse at *C*. Slowing of the rhythm occurs in both cases. The most effective inhibitory stimulus was found to be alternating negative and positive pressures within the segment, such in fact as are produced by the respiratory efforts after closure of the tracheal outlet in either the inspiratory or the expiratory phase.

Between the arrow marks below the stethograph record in Graph II deflation of the lungs was produced by negative intrapulmonary pressure;

during the period of reflex acceleration resulting from deflation of the lungs, the tracheal wall was collapsed (at *C* in Fig. 3) and an immediate and well-marked slowing occurred, the acceleration returning on the release of the intratracheal pressure.

Graphs III and IV are from another animal. Graph III shows the reflex slowing accompanying the collapse of the tracheal wall; Graph IV the immediate slowing of the rhythm resulting from collapse of the trachea during a period of accelerated breathing accompanying deflation of the lungs.

The observation is similar to that shown in Graph II. The negative pressure used to collapse the tracheal segment was considerably in excess of that which would have been required to cause complete deflation of the lungs.

The results obtained show that pressure changes within the trachea or distortion of its walls, and presumably also of the bronchi, without any corresponding change in the volume of the lungs, have a reflex inhibitory influence on the respiration rhythm, the depth of inspiration being either unchanged or very slightly increased.

The close similarity between the result seen in these observations and the effects produced by suction of air from the air passages with negative pressure in excess of that required to deflate the lungs is evident, and suggests that the cause of the reflex slowing is identical in the two cases, namely the stimulation of inhibitory vagal nerve endings in or around the larger air passages.

It seems possible that the inhibitory stimuli giving rise to the reflex effects illustrated in Figs. 1 and 3 correspond to the electrical changes observed in the vagus by Adrian [1933], which accompany traction on the trachea and are thought by him to be due to the excitation of vagus nerve endings in the tissues near the roots of the lungs. Keller and Loeser [1930] described electrical changes in the vagus accompanying sudden suction of air from the trachea after the lungs had been completely deflated. The impulses giving rise to the reflex slowing which we have described under similar conditions no doubt correspond to the electrical changes found by these observers.

Section 3. *Reflex slowing of the respiratory rhythm consequent on closure of the tracheal outlet*

Slowing of the rhythm and increased depth of breathing occurs constantly in anæsthetized or decerebrate animals on closure of the air outlet at the height of inspiration or during the expiratory pause.

This reflex was first described by Head [1889] and is illustrated in Plate I, Graphs 8 and 9, of his paper.

The cessation of respiration in the conscious human subject on closure of the air outlet in either the inspiratory or expiratory phase with gradually increasing expiratory or inspiratory tonus respectively, described by Haldane & Mavrogordato [1916], is a phenomenon which seems to be related to consciousness or at least to the action of centres above the mid-brain; it is not seen in anæsthetized animals.

The slow rhythm, or more commonly the prolonged pause, which accompanies closure of the tracheal orifice at the height of inspiration is clearly the normal Hering-Breuer reflex to sustained expansion of the lungs and need not be further considered.

On the other hand, the slowing coincident with closure during the expiratory pause evidently cannot be so explained, the volume of the lungs remaining at the level of the base line of expiration. It has, in our opinion, the same reflex origin as the slowing described above as resulting from suction from the trachea with complete deflation or from pressure changes within a segment of the trachea. (Sections 1 and 2 of this paper.)

The slow rhythm with closure in either phase is abolished by vagotomy (cervical); it is therefore of vagal reflex origin. Fig. 4 illustrates the reflex effect on the breathing of closure of the tracheal outlet.

Method. The bellows record is introduced in Graphs A and B to indicate the point at which the closure and opening of the outlet took place, by clamping the rubber tube between the trachea and the valves, the continuous record of respiration being by the diaphragmatic slips (line 3). The manometric record made with a thin rubber tambour shows the oscillations of the intratracheal pressure. This did not exceed 20 mm. Hg in Graph C.

Graph A in the figure shows the well-marked slowing of the rhythm and increase in the amplitude of the diaphragmatic slip contractions accompanying closure of the tracheal outlet at the base line of expiration. In Graph B closure at inspiration was accompanied by inhibition of the rhythm, a prolonged expiratory pause. The rise of intratracheal pressure seen in this record is not an active effect, but represents merely the elastic recoil force of expiration. In the observation recorded in Graph C the lungs were deflated by a negative intrapulmonary pressure of about −7 mm. Hg and the trachea was closed in the expiratory phase; the immediate slowing of the accelerated rhythm is very evident. The inhibitory stimulus, whatever its origin, at once dominated the augmentor reflex effect of deflation.

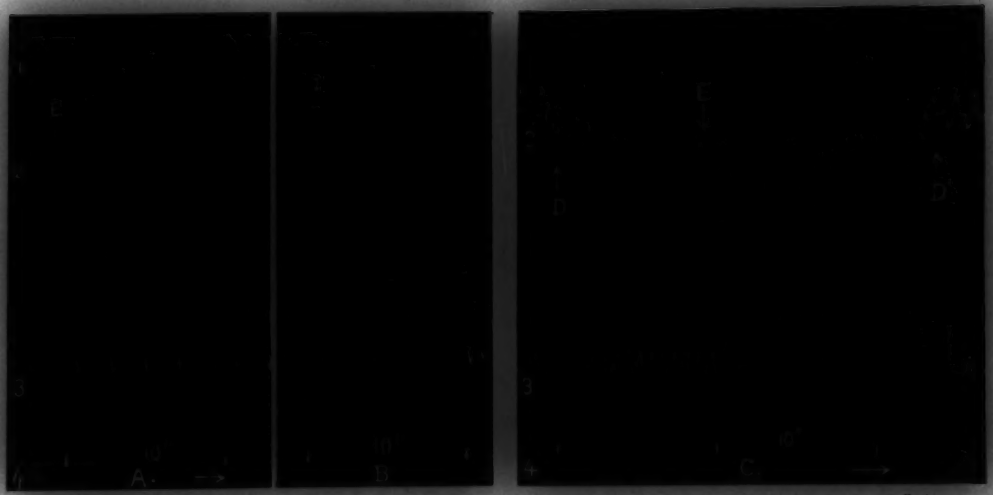

Fig. 4. Reflex effect on breathing of closure of the tracheal outlet. Rabbit, anæsthetized with medinal. Graphs A and B from one animal, Graph C from another. Line 1 of record: bellows record of ventilation from tracheal cannula. Line 2: intratracheal pressure; rubber membrane tambour. Line 3: diaphragmatic slip record. Line 4: time, 10 sec.

Graph A. Effect of closure of the tracheal outlet (clamping of rubber connexion between tracheal cannula and valves) at the base line of expiration; closure at *E*.

Graph B. Closure of outlet at end of inspiration at point *I* on the graph.

Graph C. Deflation of the lungs between the marks *D* and *D'* with closure of the outlet at *E* at the base line of expiration.

In lines 1 and 2, inspiration down. In line 3 (slip), up. The downward movement of the manometer record (2) accompanies inspiration, and represents a negative intrapulmonary pressure in Graph A of about 10 mm. Hg: in Graph C, owing to slackness of the rubber membrane, the pressure record is incomplete.

The close resemblance between the effects illustrated in Fig. 4, Graph C, and in Fig. 1, Graph 3, suggests that the reflex origin is identical in the two cases.

The condition established by the closure of the air outlet in either phase may briefly be considered here. With closure at inspiration the lungs are maintained in a state of expansion at the inspiratory level. The reflex cause of the inhibition seen in Graph B is evident. With closure at expiration the respiratory efforts continue, a negative intrapulmonary pressure of about −10 mm. being produced at each inspiration in observation A and −20 mm. in C. The actual expansion of the lungs corresponding to these pressures, which would amount in A to a little more than 1 p.c. of the total volume, is too small to account for the reflex slowing. There are however three possible sources of excitation: (*a*) Some distortion of the extrathoracic air passages will result from the pressure changes. (*b*) As already mentioned, the vigorous inspiratory efforts, although they cannot produce any significant change in the pulmonary volume, will set up a condition of tension or stress in the tissues of the lungs and their surroundings. (*c*) The most evident effect, seen in Graphs A and C, is the considerable pressure oscillation within the lungs and air passages.

Section 4. *Arrest of the respiratory movements at different phases of respiration by fixation of the thoracic walls*

To determine (*a*) what, if any, effect arrest of respiratory movements at any given point in the cycle might have, when no change in the volume or in the shape of the lungs or air passages or of air pressure within them occurs, or (*b*) whether under these conditions pressure changes as such have any reflex effect, the following method has been employed.

Method. The animal was enclosed in a chamber similar in principle to the air chamber we have previously described [1928], the essential difference being that the space around the animal was completely filled with water in place of air, the animal being therefore surrounded by an incompressible medium.

The chamber was a large glass jar sufficiently deep and with a large enough neck to allow the introduction of a 1·5 kg. rabbit.

Through the cover, a waxed bung, passed a number of tubes: (*a*) A tracheal connexion. Immediately outside the chamber this was connected by a side tube to a mercury manometer attached through valves to an air reservoir with bellows recorder. (*b*) A tube connected to a Hürthle manometer (8 mm. membrane) registering the changes of pressure in the space surrounding the animal. (*c*) A curved tube passing

to the highest point in the chamber through which air could be completely evacuated when the chamber was filled with water. (*d*) A tube, 15 mm. in diameter, connected through a rubber tube to a water reservoir (a 5 litre bottle with a tubulure near the bottom). This reservoir is essential to allow breathing to take place, the water flowing to and from the chamber with the respiratory movements. The surface level of the water in the reservoir was adjusted to the mean level of the thorax of the animal within the chamber, the normal volume of the thorax being thus maintained. A bellows recorder was connected to the air space above the water in this reservoir, enabling a plethysmographic record of the breathing to be obtained.

The rubber tube connecting the reservoir to the chamber could be clamped at any desired phase of the breathing, the movements being thus immediately arrested without any change in the intrapulmonary pressure or volume of the lungs, the muscles contracting isometrically.

The record of the respiratory efforts during fixation are registered by a Hürthle manometer connected to the water-filled space, each inspiration producing a rise in pressure corresponding to its force, the change of volume due to the small elastic manometric membrane being negligible.

Fig. 5 illustrates the results of observations carried out by this method. It will be seen in observation A, that at *I*, where the water outlet was clamped at the level of inspiration, the thorax thus being fixed in a position of normal expansion, inhibition of the breathing took place. This inhibition is evidently the normal Hering-Breuer reflex. At *E*, where the outlet was clamped at the expiratory level, there is no slowing such as would have occurred if the tracheal outlet had been closed, but a slight and transitory acceleration.

The very slight undulations shown on the record of the bellows volume recorder connected to the trachea (Fig. 5, line 2) shows how effectively the method of fixation has prevented any significant change in the volume of the thoracic contents. The presence of air in the gastro-intestinal contents probably explains why complete rigidity cannot be attained. The suppression of all changes of pressure and the reduction of volume changes to a minimum has therefore abolished the reflex inhibition observed in other conditions. It is of interest that, although the discharge from the centre continues and is augmented in strength, this has no effect on the rate of discharge.

In observation B very considerable changes of intrapulmonary pressure were produced either by suction of air from the trachea or the reverse. At *I*, during a period of arrest at the level of inspiration, a

negative intrapulmonary pressure of −40 mm. Hg was produced. Apart from a few inspiratory efforts, arising probably from the slight diminution of the volume of the lungs which is almost unavoidable, no significant effect resulted.

At *E* and *E'* during succeeding periods of arrest at the expiratory level, first a positive intrapulmonary pressure of +20 mm. and at *E'* a negative pressure of −20 mm. Hg were produced. In neither was any significant reflex effect seen.

Fig. 5. A. Effect of arresting the respiratory movements by fixation of the thorax. B. Effect of intrapulmonary pressure changes during such fixation.

Observation A. Rabbit, 1900 g. Medinal. Line 1: bellows connected to air space above water reservoir outside chamber, recording respiration plethysmographically. Line 2: bellows connected with tracheal outlet. Line 3: Hürthle manometer registering pressure changes in water space round animal. Line 4: time, 10 sec.

Observation B. Rabbit, 1500 g. Medinal. Line 1: plethysmographic record of respiration. Line 2: Hürthle manometer record of pressure changes in water surrounding animal. Line 3: Hg manometer record of intratracheal pressure. Line 4: time, 10 sec.

It seems clear that, although the alveoli as well as the air passages are equally affected by the pressure changes, these in themselves have no excitatory effect. It may be concluded therefore that the reflex slowing seen with closure of the tracheal outlet or suction from the trachea under ordinary conditions must be related to distortion or tension affecting the air passages or other intrathoracic tissues.

Discussion

The observations recorded in this paper have been briefly discussed in connexion with the sections to which they belong.

The experimental evidence adduced in Section 4 can be accepted as proving that pressure changes unaccompanied by changes in the volume of the lungs or air passages have no excitatory effect on the vagus terminations, and that therefore any reflex slowing or acceleration of the respiratory rhythm must be due to changes in the shape or stress to which the lungs, the air passages or their surroundings are subjected. That the reflex effects described are of vagal origin is shown by the fact that they do not occur after section of the cervical vagi.

Note. The path of the afferent fibres of the vagus conveying the inhibitory impulses from the trachea is not accurately known. Symington [1914], quoting Kandarsi, states that in the dog and cat the upper half of the trachea is supplied by branches of the superior laryngeal nerve; that impulses passing along such branches are not the sole cause of the reflex slowing seems, however, to be shown by the fact that this reflex still occurs after the trachea has been firmly ligatured below the larynx. Lemere [1932] describes the nerve supply to both the intrathoracic and cervical segments of the trachea in the dog as arising from a branch of the recurrent laryngeal nerve. For the reference to Lemere's paper we have to thank Miss Fielding of the Anatomical Department, University College, London.

The site from which the excitatory impulses responsible for the slow rhythm accompanying suction when the lungs are completely collapsed must evidently be related to the air passages and not the alveoli. The similar slowing accompanying closure of the tracheal outlet in the expiratory phase must be regarded as of identical origin. It is clear that the stimulus might equally well arise in the walls of the trachea and bronchi or from changes in the tension of the mediastinal or other neighbouring tissues. It might be thought that the observations on the tracheal segment (Section 2) indicate that the nerve endings excited must be situated in the wall of the trachea: there is, however, with the procedure employed, a short intrathoracic portion of the trachea the collapse or distension of which would affect also the mediastinal tissues.

We have hitherto been unable to obtain satisfactory evidence of reflex slowing by suction from the trachea, with the chest open, in dogs after the rhythmical contractions of the diaphragm have been re-established during a pause in the artificial respiration. This would certainly suggest that the stimulus arises entirely or mainly from tissues external to the air passages. We are not, however, convinced that the conditions existing with the open thorax permit of any such definite conclusion without further investigation, which we propose to carry out.

A question of considerable interest which is also left for later consideration is as to whether the augmentor fibres described in our previous communications originate only in the alveoli, or whether they also arise in the same situations as the fibres carrying the reflex impulses described in this paper.

Summary

1. The reflex slowing with high intrapulmonary negative pressures is shown not to occur until complete collapse of the lungs has taken place, and that it is therefore not of alveolar origin. A means of rigid fixation of the diaphragmatic slips of the rabbit in Head's method of recording the respiratory movements is described.

2. A method is described of investigating the effect of pressure changes in the trachea without interference with respiration. It is shown that either distension or collapse of the wall of the trachea leads to reflex slowing of the respiratory rhythm.

3. The cause of the reflex slowing accompanying closure of the tracheal outlet in the expiratory phase is discussed.

4. A method is described of fixation of the thoracic wall by which the respiratory movements can be arrested at any desired point in the cycle without any change in the intrapulmonary pressure. It is shown that pressure change as such has no reflex effect on the respiratory rhythm.

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SPECIES VARIATION IN THYROTROPIC ACTIVITY OF THE PITUITARY GLAND

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(*Received* 22 *July* 1936)

It was shown by Hill [1934], who extended the results of other authors, that the pituitary glands of different species vary greatly in their content of gonadotropic hormones. Following the quantitative work of Rowlands & Parkes [1934] on thyrotropic extracts of ox pituitary, it seemed desirable to make a comparative study of similar extracts from the pituitaries of other species and to correlate the results obtained with those of Hill for the gonadotropic hormones.

Loeb & Bassett [1930] noticed that the relative response of the reproductive organs and the thyroid of the immature guinea-pig depended on the type of pituitary injected. Rabbit and guinea-pig pituitaries caused follicular maturation with ovulation, but very little hypertrophy of the thyroid. The ox pituitary, on the other hand, was highly thyrotropic, but failed to cause ovulation. This work was extended later by Loeb [1932], Loeb *et al.* [1935] and Kunkel & Loeb [1935], whose conclusions will be discussed later in the light of the results described. Bates *et al.* [1935] have compared the thyrotropic and follicle-stimulating activities of pituitaries under different conditions. Loeb [1932] and Greep [1933] associated thyrotropic activity with the luteinizing hormone of the pituitary, but later Greep [1935] succeeded in separating the thyrotropic substance from this gonadotropic hormone.

Material

Pituitaries were obtained from five different species, ox, horse, sheep, pig and dog. They were desiccated in acetone and extracted once with 10 vol. of *N*/20 sodium hydroxide in cold store overnight. The extracts were neutralized, centrifuged and the supernatant fluid precipitated by the addition of 5 vol. 96 p.c. alcohol and 1 vol. ether. The precipitate was

filtered and weighed. The amount extracted and the yields obtained from the different species are given in Table I. Batches of cow, sheep and pig pituitaries were also extracted with 25 vol. of 50 p.c. aqueous pyridine after desiccation, the subsequent procedure being the same as the above. The yields are shown in Table III.

TABLE I. Yield of thyrotropic extract obtained by alkali extraction of acetone desiccated pituitaries of various species

Species	Amount extracted g.	Yield g.	Yield p.c.
Cow (T 19 B)	100	10·05	10·0
Horse	5	1·27	25·5
Sheep	1·25	0·17	13·5
Pig	5	0·97	19·5
Dog	0·5	0·071	14·0

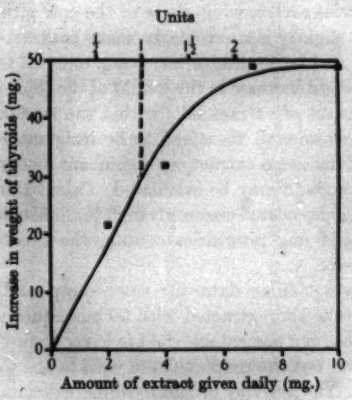

Fig. 1.

Immature female guinea-pigs, weighing about 200 g., were used for assay of the thyrotropic hormone. Injections were made subcutaneously, once daily, for 5 days. Five guinea-pigs were used in each group; they were killed 24 hours after the last injection and weighed. The thyroids were dissected out and, after fixation in aqueous Bouin's fluid, weighed from 70 p.c. alcohol on a torsion balance. The weight of the thyroids above normal was used to indicate the response to the treatment. The normal thyroid weight for the mean body weight at autopsy was calculated from the linear regression given by Rowlands & Parkes [1934],

6 g. difference in body weight being equivalent to 1 mg. difference in weight of thyroids.

The units of thyrotropic activity are calculated from the standardization curve of Rowlands & Parkes, which, corrected by the above method for variation in body weight, is given in Fig. 1.

Results

It can be seen from Table I that there is a considerable species variation in the yields of thyrotropic extract, there being about 2½ times as much alkali-soluble material in the horse pituitary as in that of the cow. Since the loss of water on desiccation is not materially different in the five species, this difference must be reflected in the fresh gland.

The results of the assay are given in Table II. The extract of the dog pituitary is the most active, while those of the cow pituitary and of the pig pituitary are slightly less active. In sharp contrast to these are the extracts of sheep and horse pituitaries; 4 mg. daily of the latter extract gave only a very slight increase in the weight of the thyroids. The increase given by this amount of extract falls within the standard error[1] of the response and is considered, therefore, to be insignificant. The increase given by 4 mg. of the sheep extract is a significant increment from which the activity of the gland may be calculated. Calculation of the number of units per gram of desiccated tissue, given in the final column of Table II, shows that pig and dog pituitaries contain the greater quantities of thyrotropic hormone.

Table III gives similar data for cow, sheep and pig pituitaries desiccated in acetone and extracted with 50 p.c. aqueous pyridine.

In each instance the percentage yield is lower than that obtained by alkaline extraction, but the proportionate yield of the three species does not vary greatly. The activity of the pyridine extracts is greater than that of the alkali, indicating that a more concentrated extract is obtained by the use of this solvent. A similar result was obtained by Rowlands & Parkes [1934] on the cow pituitary.

Aron & Benoit [1932] have suggested that excessive œstrin may inhibit thyroid activity and since the horse pituitary extracts contained much gonadotropic activity, the tests of the thyrotropic activity of these extracts were repeated on ovariectomized guinea-pigs. It can be seen that the results shown in Table IV do not differ much from the data

[1] The standard error of the curve shown in Fig. 1 in which ten animals were used in all the experimental groups is ±3·6. For the control group only five animals were used, giving a standard error of ±5·4.

TABLE II. Activity of thyrotropic extracts, prepared by alkali extraction of the pituitaries of various species, assayed on the immature guinea-pig

Species	Yield p.c.	Mean body weight at autopsy of test animals g.	Weight of thyrotropic extract given daily mg.	Weight of thyroids (mg.) Actual	Weight of thyroids (mg.) Above normal	Weight of unit mg.	Units per g. of extract	Units per g. desiccated powder
Cow (T 19 B)	10·0	201	7	71·0	38·2	5	200	20
Horse	25·5*	233	4	39·2	3·2	50†	20†	5†
Sheep	13·5	216	4	40·4	7·1	16	62	8
Pig	19·5	200	4	56·2	25·4	5	200	40
Dog	14·0	162	2	41·2	16·5	4	300*	43

* Corrected for anterior pituitary only.

TABLE III. Activity of thyrotropic extracts, prepared by pyridine extraction of the pituitaries of cow, sheep and pig, assayed on the immature guinea-pig

Species	No. of extract	Yield p.c.	Amount given daily mg.	Increase in weight of thyroids mg.	Weight of unit mg.	Units per g. of extract	Units per g. of desiccated powder
Cow	AP 15 B	8·5	4	37	3	330	28
Sheep	AP 11 B	9·5	7	18	6	170	16
Pig	AP 12 B	16·5	4	37·6	3	330	54

given in Table II, so that coincident stimulation of the ovaries does not appear to be an interfering factor. Hill [1934] found that, using the rabbit-ovulation test, gonadectomy of the donor decreased the gonadotropic activity of the pituitary, while other authors (quoted by Hill), using the immature rat or fowl as test animals, obtained directly opposite results. Table IV, however, shows that castration of the horse does not appear to influence the amount of thyrotropic hormone contained in the pituitary.

TABLE IV. Activity of thyrotropic extract of horse pituitary assayed on the ovariectomized guinea-pig

Source of pituitary	No. of extract	Yield p.c.	Amount given daily mg.	Increase in weight of thyroids mg.
♂ ♀ ♂	AP 2 B	26·5	4	−0·8
− − ♂	AP 3 B	18·5	4	−1·5

Striking differences were found by Hill [1934] in the gonadotropic activity of the pituitaries of different species according to whether they were tested on œstrous rabbits or on immature rats. The results obtained for the thyrotropic hormone are compared with Hill's results in Table V.

TABLE V. Comparison between species variation in gonadotropic and in thyrotropic activity of the anterior pituitary

	Units per gram desiccated powder		
	Gonadotropic		Thyrotropic
Species	Œstrous rabbits	Immature rats	Guinea-pig
Horse	1230* (9)	150	75 ?(1)
Pig	1560 (9)	17	40 (2)
Sheep	1865 (11)	60	8 (2)
Dog	1230 (9)	—	43* (2)
Cow	160 (1)	1	20 1

* Corrected for anterior pituitary only.

Species variation in the gonadotropic and thyrotropic activity of the pituitary (cow pituitary = 1) is shown by the figure in brackets. It is evident that, whereas the horse pituitary contains very large amounts of follicle-stimulating hormone compared with that of the cow, the latter contains at least four times as much thyrotropic hormone. The pig pituitary, which is also more active on the rat ovary than the cow pituitary is, contains also twice as much thyrotropic activity. Again, the sheep pituitary contains a high proportion of both gonadotropic hormones but only a very small amount of thyrotropic hormone. Unfortunately the

amount of dog pituitary extract did not permit of an assay on rat ovaries, but Table V shows that it contains twice as much thyrotropic hormone as that of the cow.

Discussion

Loeb and his co-workers [1935] tested extracts of pituitaries of various species on the immature female guinea-pig and found that those of cattle, sheep and pigs fall into one class since they all caused atresia of the follicles and some slight formation of atretic corpora lutea. Marked thyroid hyperplasia was obtained with the pituitaries of all three species. Into this class Kunkel & Loeb [1935] placed the human pituitary, although the degree of thyrotropic response was rather less and also more variable. The pituitaries of the rat, cat and rabbit, on the other hand, caused growth of the follicles, and ovulation with formation of corpora lutea; in these species the thyrotropic activity of the pituitary was slight. They conclude that possibly a direct relationship exists between the luteinizing and the thyrotropic hormones and an inverse ratio between the follicle-stimulating and the thyrotropic hormones. The present results show that there appears to be no regular relationship between the amount of the luteinizing and of the thyrotropic hormones in the pituitary of the species examined. On the other hand, when the amount of follicle-stimulating hormone in the pituitary of any of these species is high, the thyrotropic content is low, that is, the follicle-stimulating hormone in units per gram of desiccated powder varies inversely as the thyrotropic activity of the gland.

Bates *et al.* [1935] found a direct relationship between the follicle-stimulating and the thyrotropic activity of the pituitaries of various types of cattle, i.e. embryos, immatures, steers, bulls and pregnant cows.

On the view of Collip [1934] that the responsiveness of an animal to an injected hormone varies inversely with the hormone content and production of the animal's own gland, it would appear from these results that the horse and the sheep should react intensely to thyrotropic hormone because of the low content of this hormone in the pituitary of these species, such as Loeb & Bassett [1930] found for the guinea-pig and the rabbit.

Summary

Extracts of the pituitaries of five species, viz. ox, horse, sheep, pig and dog, have been assayed for their thyrotropic activity, by the method described by Rowlands & Parkes [1934]. In descending order of

potency, measured in units per gram of desiccated tissue, these species fall as follows: dog, pig, cow, sheep and horse.

In comparison with their gonadotropic activity, it was found that an inverse relationship existed between follicle-stimulating and thyrotropic activity. No direct correlation existed between the luteinizing and the thyrotropic hormones.

On the basis of Collip's generalization, the horse and the sheep should respond well to thyrotropic extracts.

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612.621:615.363.814.3

INHIBITION OF OVULATION IN THE RABBIT BY ANTI-GONADOTROPIC SERUM

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(*Received* 22 *July* 1936)

It has been shown by Selye *et al.* [1934*a*], Hertz & Hisaw [1934], Fluhmann [1935*a*, *b*], Hisaw *et al.* [1936] and other authors that prolonged administration of gonadotropic preparations of the pituitary body leads, after an initial period of stimulation, to insensitivity of the animal. In the same way rats become resistant to prolonged injection of gonadotropic preparations of urine of pregnancy [Selye *et al.* 1934*b*] or urine of teratoma testis cases [Twombly & Ferguson, 1934]. Collip and his co-workers [Bachmann *et al.* 1934] have shown that this decrease of sensitivity is due to the production of anti- or inhibitory substances in the blood of these animals, and that insensitivity can be conferred on an untreated animal by injection of serum from a desensitized one.

There is considerable confusion as to the specificity of the immunity produced by different gonadotropic preparations. Selye *et al.* [1934*a*] have shown that the rats protected by prolonged injection of gonadotropic preparations of the pituitary respond to urine of pregnancy extracts and vice versa, demonstrating that the anti-substances produced by these substances are not identical. Meyer & Gustus [1935] have recorded also that the anti-gonadotropic substance evoked in the rhesus monkey by the gonadotropic substance of pregnant mare serum does not inhibit human pituitary or urine of pregnancy extracts or sheep pituitary preparations. Further work on the specificity of this anti-gonadotropic substance has been described by Fluhmann [1935*b*].

Most of this work has been performed on the immature female rat. Apart from the single experiment of Bachmann *et al.* [1934] nothing appears to have been done on the œstrous rabbit. The rabbit, ovulating only after copulation, has been shown by numerous authors to be a very suitable test-animal for the assay of gonadotropic preparations and it

therefore appeared equally suitable for the assay of anti-gonadotropic sera. In practice, this expectation was fulfilled [Parkes & Rowlands, 1936], and the present paper records work on the inhibition of naturally and artificially stimulated ovulation in the rabbit by the administration of anti-gonadotropic serum.

Technique

A number of male and female adult rabbits were injected subcutaneously with 25 mg. daily of an extract obtained by alcoholic precipitation of a 50 p.c. aqueous pyridine extraction of ox anterior pituitary that had been dried with acetone. After 10 weeks or more, 20 c.c. of blood was withdrawn once weekly from the marginal ear vein of each rabbit. The serum was obtained by allowing the blood to clot at 37° C. for 1 hour and subsequently by compression of the clot; it was stored at −2° C.

The activity of the serum was tested by its ability to prevent ovulation in injected or mated œstrous rabbits. The serum was injected intravenously within 15 min. of mating or of the injection of gonadotropic extract, except in the experiments described on p. 310. Each assay group contained ten œstrous rabbits and the unit (Rb. u.) has been considered as the amount of activity required to cause or inhibit ovulation in 50 p.c. of such a group [Hill *et al.* 1933].

The rabbits were subjected to an exploratory laparotomy under aseptic conditions and ether anæsthesia twenty-four hours after the injection.

Inhibition of ovulation in œstrous rabbits given gonadotropic preparations

Inhibition of the gonadotropic action of the antigenic extract

Our first experiment was designed to show whether the serum from rabbits after prolonged injection of pyridine extract of anterior lobe would inhibit the ability of the same extract to cause ovulation in the œstrous rabbit. At the beginning, therefore, a dose/response curve was made for the ovulation-producing power of the extract. The results are shown in Fig. 1, curve I. A similar dose/response relation was then worked out giving 0·5 c.c. of the serum to each rabbit with the gonadotropic extract (curve II). The shift of the curve to the right shows that nearly 3 mg. more extract is required to produce the same result when 0·5 c.c. of serum is also given. This result was confirmed by the repetition of the experiment with double the amount of the serum, which still further increases the amount of extract required (curve III).

The amount of the extract required to cause ovulation in 50 p.c. of the rabbits is about 0·75 mg. when no serum is given, 3·5 mg with 0·5 c.c.

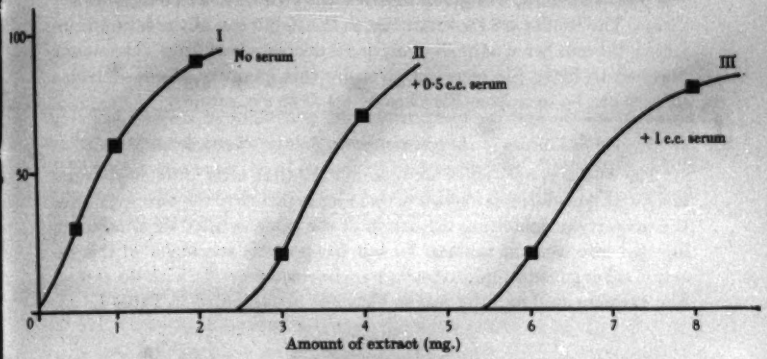

Fig. 1. Dose/response curve for ovulation-producing extract, with and without anti-gonadotropic serum.

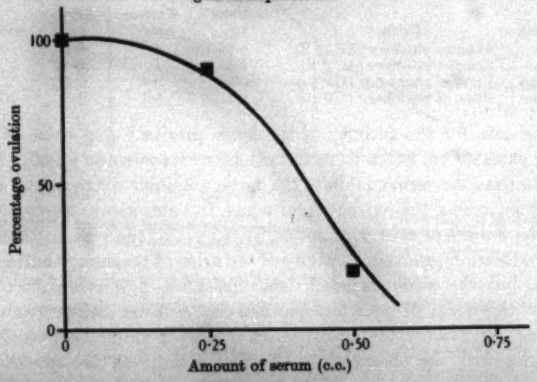

Fig. 2. Ovulation-producing power of 3 mg. extract given with varying amounts of anti-gonadotropic serum.

serum, and 6·75 mg. with 1·0 c.c. serum. 1 c.c. of serum will thus neutralize about 6 mg. of the extract.

The same problem was then approached in a slightly different way, and the amount of serum required to "neutralize" the ovulation-pro-

ducing power of a constant dose of the antigenic extract was worked out. 3 mg. of the extract, a dose known to cause ovulation in practically 100 p.c. of animals, was given together with 0·25 c.c. and 0·5 c.c. of the serum. The results are shown in Fig. 2. When 0·5 c.c. of the serum is injected, the number of animals ovulating is decreased to 20 p.c. The amount required to halve the response given by this quantity of gonadotropic extract can be seen from Fig. 2 to be 0·4–0·45 c.c. serum.

Inhibition of the gonadotropic effect of other extracts

The above results show quite definitely that after prolonged treatment with gonadotropic extract of ox anterior pituitary the serum acquires the property of inhibiting the action of the same extract when both are injected into œstrous rabbits. To test the possible specificity of this reaction other gonadotropic extracts were injected together with the serum. The extracts used and the results obtained are recorded in Table I.

TABLE I.

Species	Extract	Amount for 50 p.c. ovulation in rabbits mg.	Given with serum: Extract mg.	Serum c.c	p.c. ovulating
Ox	Anterior pituitary (AP 15 B)	0·75	4	0·5	70
Horse	Anterior pituitary (AP 2 B)	1·25	4	0·5	10
Human	Urine of pregnancy (UP 10)	0·2	0·4	0·5	80
Human	Urine of pregnancy (UP 10)	0·2	0·4	1	10

The data for the activity of the horse pituitary and urine of pregnancy extracts are taken from the results of Rowlands [1936]. It will be seen that the serum inhibits the horse pituitary extract just as efficiently as it does the extract from which the anti-serum was prepared; roughly, 3 units of each is inhibited by 0·5 c.c. of the serum. Inhibition of the ovulation-producing activity of the urine of pregnancy extract also occurs, but the serum is much less efficient in protecting the ovaries against this form of gonadotropic activity, 1·0 c.c. being required to decrease appreciably the effectiveness of only 2 units. This result does not agree with the observations of other authors on the specificity of anti-gonadotropic activity. Further work, however, is being carried out using the immature rat as the test animal, and the results so far show that the serum raised against pituitary extract does cause some inhibition of the activity of urine of pregnancy extracts.

The serum raised against pyridine-treated ox anterior lobe is thus capable of reacting with a variety of other chemically treated gonadotropic preparations. Our next experiment was to test the serum against

saline extract of fresh untreated anterior lobe of ox and sheep, and definite inhibition was obtained of the ovulation-producing activity of saline extracts of the untreated gland. The interaction of hormone and antiserum is thus not dependent on denaturation of the extract.

Inhibition of ovulation in the mated rabbit

The success in inhibiting the ovulation-producing action of saline extract of fresh sheep pituitary suggested that the serum might inhibit the gonadotropic action of an animal's own pituitary. The rabbit, which normally ovulates only when the pituitary is stimulated by mating, offered an excellent test of this possibility.

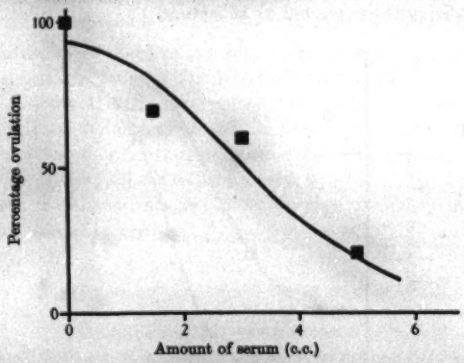

Fig. 3. Effect of various amounts of serum on the percentage of rabbits ovulating after mating.

A large number of rabbits, all of the same breed, were mated. Ten rabbits were not given serum, and two were given 10 c.c. of normal rabbit serum; all these ovulated. The remainder were given various quantities of anti-serum intravenously 5–15 min. after mating. The results of this experiment are given in Fig. 3. When 1·5 c.c. of serum is injected, there is a decrease to 70 p.c. ovulating, the percentage falling as the amount of serum injected is increased. Of ten rabbits given 5 c.c. of serum, only two ovulated, an inhibition of 80 p.c. Two others injected with 10 c.c. both failed to ovulate. Dr Hammond kindly tested this serum and found that the injection of 10 c.c. into each of five mated rabbits uniformly prevented ovulation—a result entirely in keeping with

our own. There is no evidence that this result was due to interference with secretion by the pituitary, and anti-gonadotropic serum produced by the rabbit in response to chemically treated ox pituitary extract must therefore be capable of passively immunizing another rabbit against the ovulation-producing substance of its own pituitary.

From Fig. 3 it will be seen that about 3 c.c. of the serum is required to inhibit ovulation in 50 p.c. of mated rabbits. According to Hill [1934] the mated rabbit produces an excess of gonadotropic substance over that actually required to cause ovulation, possibly 5–10 Rb. units in all. 3 c.c. of the serum, however, would neutralize 25 units of the antigenic ox pituitary extract, and it is likely, therefore, that the serum is less efficient in neutralizing the rabbit's own gonadotropic substance than it is in neutralizing the extracted ox substance.

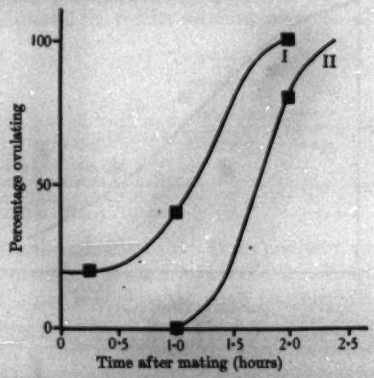

Fig. 4. Effect of time of mating at which anti-gonadotropic serum is given. Curve I, 5 c.c. of serum given; curve II, 10 c.c. of serum given.

Effect of anti-gonadotropic serum given at different times after mating

It is now well known that the pituitary body secretes an effective amount of the ovulation-producing hormone within 1 hour after mating, but it has not previously been possible to estimate the time that elapses before irreversible changes take place in the follicles. By giving serum and thus neutralizing the circulating gonadotropic hormone at different times after mating, it has been possible to make such an estimation. The results given in Fig. 4 show that even a large amount of serum is powerless

to inhibit ovulation in most rabbits if administration is left until 2 hours after mating. This result is not accounted for by the continued secretion of the pituitary in the second hour after mating, because such secretion presumably takes place equally when the serum is given early, so it may be ascribed to the setting in of irreversible changes in the follicle within 2 hours after mating.

Summary

1. Anti-gonadotropic serum prepared by prolonged injection of rabbits with chemically treated ox anterior pituitary extract inhibits the ovulation-producing activity of

(*a*) the antigenic extract itself;
(*b*) similar extracts of horse pituitary;
(*c*) saline suspension of fresh cow and sheep anterior pituitaries.

The serum is less effective in inhibiting the activity of human urine of pregnancy extracts, but it is far from ineffective.

2. This serum, given intravenously to rabbits immediately after mating, inhibits the ovulation which would otherwise occur in 10–12 hours, and may therefore be said to effect passive immunization against the secretion of the rabbit's own pituitary.

3. There is thus no suggestion of species specificity in the anti-ovulation action of this serum.

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612.627:612.018

THE EFFECT OF CERTAIN HORMONES ON THE ACTIVITY OF THE UTERINE MUSCLE OF THE GUINEA-PIG

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(*Received* 24 *July* 1936)

Removal of the corpus luteum activity during pregnancy is followed by different results according to the species investigated. In some species, such as the mouse and the rabbit, gestation is interrupted, whilst in others, such as the guinea-pig, the mare, the cat, and man, it may continue to full term. Hence the luteal secretion is necessary for the maintenance of pregnancy in the first series of animals, whilst in the second series the corpus luteum is not an essential organ, though possibly the luteal hormone is secreted elsewhere. Indeed the finding of this hormone by Adler *et al.* [1934] in the human placenta suggests that this organ may be another site of its production; also, the results obtained by Courrier & Gros [1935] in the cat, and by Selye *et al.* [1935] in the rat, offer strong evidence that progestin may be elaborated by the uterine contents.

The exact nature of the action exerted by the luteal hormone in the maintenance of pregnancy has by no means been worked out, but it appears possible that two alterations in the physiological activity of the uterine muscle are of significance. These effects of progestin are (1) desensitization of the muscle, i.e. inhibition of its reaction to the oxytocic hormone of the posterior pituitary lobe [Knaus, 1930; Robson & Illingworth, 1931; Robson, 1935], and (2) inhibition of the rhythmic contractions of the muscle exhibited in the living animal after injection of œstrin [Reynolds & Allen, 1932]. The first of these effects has so far been definitely observed only in the rabbit, in which the alterations in the reactivity can be seen both *in vitro* and *in vivo*. In the other rodents

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examined the luteal hormone exerts no desensitizing action on the uterine muscle *in vitro* [Siegmund, 1930], but the reactivity of the muscle in the intact animal has not yet been studied. Inhibition of the rhythmic contractions of the uterus by progestin has been investigated only in the rabbit, in which this effect is obtained *in vivo* but not on the isolated muscle [Reynolds & Allen, 1932; Robson, 1935]. The action of the hormone on the intact uterus of other animals has not yet been determined, though indirect and rather contradictory evidence has been obtained in the human subject from investigation of uterine activity at various stages of the menstrual cycle [Knaus, 1935; Moir, 1934].

The object of the present investigation was to find out whether the luteal hormone exerts any action on the uterine activity of animals other than the rabbit. Information was sought on four points, namely, (1) whether the reactivity to oxytocin measured *in vivo* is affected; (2) whether any effects can be demonstrated on the reactivity *in vitro* when this is quantitatively measured; (3) whether the rhythmic contractions recorded *in vivo* are inhibited; and (4) whether there is any effect on the spontaneous contractions *in vitro*.

In addition a gonadotropic preparation was tested to see whether the gonadotropic hormones might directly affect the uterine function, as it has been suggested [Reynolds, 1932] that they may exert a controlling influence on the uterine activity in animals in which removal of the ovaries during pregnancy does not lead to abortion.

Methods and materials

The experiments were performed on thirty-three virgin guinea-pigs about 250–300 g. in weight. All the animals were ovariectomized by the dorsal route.

Injection of œstrin was always commenced on the day following operation, except in the two uninjected controls. Crystalline œstrone in solution in oil was used.

The following preparations of corpus luteum hormone were given: (1) crude extract made from bovine corpora and referred to in Table I as "own"; 1 kg. of corpora yielded some 60 c.c. of this extract, about 7 c.c. of which gave full progestational proliferation, desensitization of the muscle, and inhibition of the uterine motility in the mature rabbit; (2) purified preparations in solution in oil received from Organon Laboratories, standardized on the immature rabbit; (3) crystalline progesterone; we are indebted for this and the purified preparation to Dr Tausk of Organon Laboratories; (4) "proluton" kindly supplied by

Messrs Schering (1 rabbit unit of proluton = 0·75 mg. progesterone dissolved in oil).

A gonadotropic preparation, referred to as M 2 in Table I, made from pregnancy urine was used, the rabbit ovulating dose of which was approximately 0·5 mg. This was administered in solution in saline.

All these injections were made subcutaneously.

The experiments were performed on four groups of ovariectomized guinea-pigs: (1) animals receiving no injections; (2) animals receiving œstrone only; (3) animals injected with œstrone and progestin; and (4) animals injected with œstrone and gonadotropic hormone.

All animals (except group 1) received the course of œstrone injections spread over three or seven days, 0·01 mg. of the hormone in 0·1 c.c. of oil being given each day morning and evening. The last injection of œstrone was in all cases made three days before the examination of the uterus. In the second group the animals thus received no injections during the 2 days preceding the experiment. In the third group progestin was given twice daily for the 3 days immediately preceding the experiment, the total amount being indicated in Tables I and II. In the fourth group gonadotropic hormone was administered twice daily on the 3 days before the experiment; in addition GP 20 and GP 21 received a dose on the day of experiment; the total amounts are also given in Tables I and II.

Following the period of injections the animals were operated on under ether anæsthesia, exept GP 25, 26, 27, 28 and 31, in which the experiment was performed under chloralose anæsthesia. In the majority of experiments one uterine horn was removed and cut in halves which were used for duplicate determinations *in vitro*. The other horn was used for the experiment *in vivo*. Two methods were used for recording the uterine activity *in vivo*. In the first the variations in the intra-uterine volume were registered, while in the second the contractions of the longitudinal muscle were recorded. The second method was elaborated with the object of obtaining a very high degree of sensitivity as it was to be used in ovariectomized untreated animals.

In the first method a small glass cannula was introduced through the vagina and cervix and tied in position in the lower part of the uterine horn. A small incision was then made in the upper part of the horn and warm saline was perfused through the cannula and allowed to escape at the incision until all the air was displaced. A ligature was then tied around the uterus immediately below the incision (see Fig. 1). The abdomen was then closed. In two animals, GP 11 and GP 13, the uterine horns were larger than usual and the caudal half only was used for the

determinations *in vivo* in order to keep the height of the expected contractions within the limits of the recording camera. Throughout the operative interference great care was taken not to disturb the blood supply of the uterus.

The uterine cannula was connected to a reservoir containing saline, there being a continuous fluid connexion. The height of the fluid in the reservoir was adjusted to give about 10 cm. of pressure of water in the

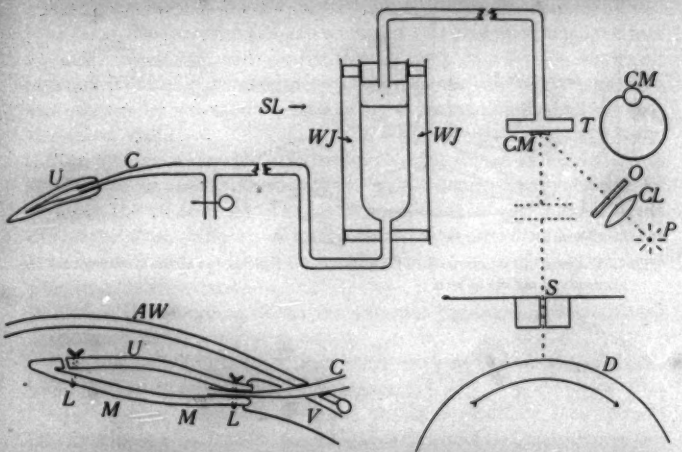

Fig. 1. Diagram of apparatus used to record movements of circular muscle *in vivo*. *U*, uterus; *C*, cannula; *M*, mesentery; *L*, ligature; *V*, vagina; *SL*, saline level; *WJ*, water jacket; *CM*, concave mirror; *T*, tambour; *P*, pointolite; *CL*, condensing lens; *O*, slit; *S*, camera slit; *D*, drum carrying bromide paper; *AW*, abdominal wall.

uterus. The upper part of the reservoir was connected to a tambour of 3·8 cm. diameter covered with a thin rubber diaphragm. A mirror was fixed on a flattened edge of the circumference of the diaphragm and optical records were taken on bromide paper on a slowly moving drum. Injection of 0·1 c.c. of fluid into the cannula produced a movement of the spot of light on the bromide paper of 2·5 cm.

In the second method the upper and lower ends of one uterine horn were tied to the extremities of a specially devised glass cannula covering the organ and thus preventing its being affected by the movements of other abdominal viscera (see Fig. 2). A thread attached to the middle of the uterus was passed through the centre of the cannula and carried over

a pulley to an isotonic lever with a mirror at its fulcrum. The abdomen was then closed over the cannula.

The beam of light reflected from the mirror fell on the cathode of a photocell (see Fig. 3). The image of the object (window *A*) was of the

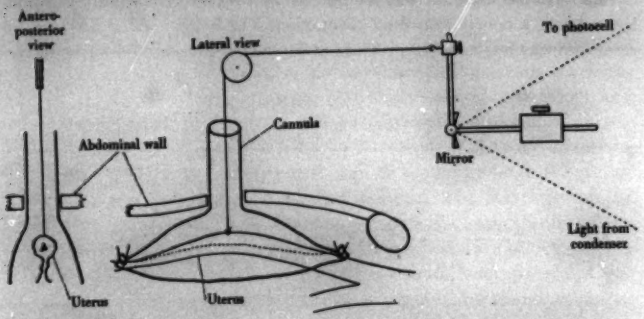

Fig. 2. Diagram of cannula used to fix uterus in the registration of the movements of the longitudinal muscle *in vivo*.

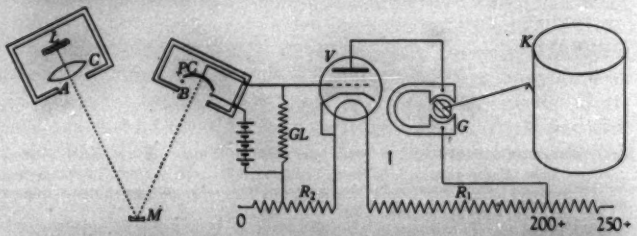

Fig. 3. Diagram of optical system with associated photocell, valve amplifier, and galvanometer writing on smoked drum. *L*, talkie exciter lamp (10 v., 7·5 amp., G.E.C.); *C*, large lantern condenser; *M*, mirror fixed to isotonic lever; *PC*, photocell CMG 8 (Osram); *GL*, grid leak (0·25–1·8 megohms); *V*, DL valve (Osram); *G*, moving coil galvanometer; *K*, smoked drum; R_1 and R_2, voltage dropping resistances connected across mains in series with cathode heater of valve; *A* and *B*, windows in front of the condenser and in front of the photocell which are both of the same shape and size as the cathode of the photocell.

same size and shape as the cathode of the photocell and rotation of the mirror led to an increase or decrease in the area of the photosensitive surface illuminated. The photoelectric current passed through a high resistance *GL* in series with a dry battery of 60 volts. The voltage drop in

GL was arranged to oppose the negative bias on the grid of valve *V* obtained from the tapping on R_2. The galvanometer *G* writing on a smoked drum was connected between the anode of the valve *V* and a tapping on R_1 at 200 volts. An increase in the area of illumination of the photocell results in an increase in the anode current of *V*, and so the pointer of the galvanometer rises up on the drum by an amount proportional to the increase in the illumination. The excursions recorded on the smoked paper are thus directly proportional to the movements of the uterus.

A large-sized moving-coil galvanometer was used and its movements were oil damped. The slow contractions of the uterus did not require an instrument of high natural frequency. The recording of small slow changes was assisted by vibrations produced by a buzzer attached to the galvanometer stand.

The system is capable of providing a high degree of magnification (1000 times or more); its sensitivity may be conveniently altered by changing the value of *GL*. The method possesses, in addition, the great advantage of providing an immediate visual record of the contractions of the muscle such as is not obtained by a system employing ordinary photographic registration.

Before the recording began the external jugular vein was exposed preparatory to intravenous injection.

The excised strips were suspended in oxygenated Ringer-Locke solution at 37·5° C. in 100 c.c. containers.

An oxytocic preparation of the posterior pituitary lobe, pitocin (kindly supplied by Dr White of Parke, Davis and Co.) was used to test reactivity both *in vitro* and *in vivo*.

In all experiments an attempt was made to estimate the smallest amount of oxytocic hormone required to elicit a definite motor effect and this was taken as a measure of the reactivity of the muscle. The initial dose added *in vitro* was usually 0·005 unit. This was decreased until the minimum effective dose was found. In the experiments *in vivo* only a limited number of injections (usually not more than two) could be made. We tried to arrange the doses above and below the threshold amounts, but this was achieved only in some of the experiments.

The spontaneous rhythmic activity both *in vivo* and *in vitro* was measured directly on the tracings in cm. and the data given in the text and Table I represent these measurements. In the experiments, however, in which the photoelectric method of recording was used the actual downward movement of the centre of the uterus was calculated and is given in mm. in Table II.

Results

(a) *In vivo*

The type of response obtained *in vivo* stands in marked contrast to the characteristic response of the guinea-pig uterus to oxytocin *in vitro*. Usually the chief effect *in vivo* was an increase in the frequency of the contraction waves with no rise of the base line. When the rhythmic contractions were initially large, then an effective dose of oxytocin caused no increase or even a decrease in the height of the individual waves. These effects are illustrated in Fig. 4, in which the response of the uterus of animal GP 15 to the intravenous injection of 0·01 unit of oxytocin is shown, and also in Fig. 5 which shows the response of the longitudinal muscle *in vivo* to the intravenous injection of 0·02 unit in animal GP 32. On the other hand, if the waves were initially small, the action of oxytocin included an increase in the height of the contractions.

An examination of the data obtained by the first method of recording (see Table I) suggests that the reactivity exhibited *in vivo* shows no appreciable differences between the three groups investigated; there is no suggestion that administration of the luteal hormone has desensitized the uterus (see Figs. 6 and 7).

Measurements of the spontaneous activity *in vivo* reveal no appreciable differences in amplitude or in frequency between the various groups of animals (i.e. guinea-pigs injected with œstrin only; those receiving œstrin and progestin; those receiving œstrin and gonadotropic hormone). If averages of the amplitudes for the various groups are taken then the figures obtained for the spontaneous activity in these groups are respectively 5·6, 5·0, and 9·0 cm.

In all but two of the experiments spontaneous contractions at approximately the maximum level began shortly after the introduction of the uterine cannula. In those two cases, however, in which only half of the horn was used for determinations *in vivo* (GP 11 and GP 13) the rhythmic contractions did not attain their full height until about an hour after the beginning of the experiments; it is to be noted that the results for spontaneous activity actually recorded photographically in these two cases have been multiplied by two for the purpose of comparison with the other figures in Table I. The results obtained on the longitudinal muscle *in vivo* (Table II) agree with those obtained on the circular muscle by the first method. It will be seen that quite marked rhythmic contractions have been obtained in one untreated ovariectomized animal (GP 35).

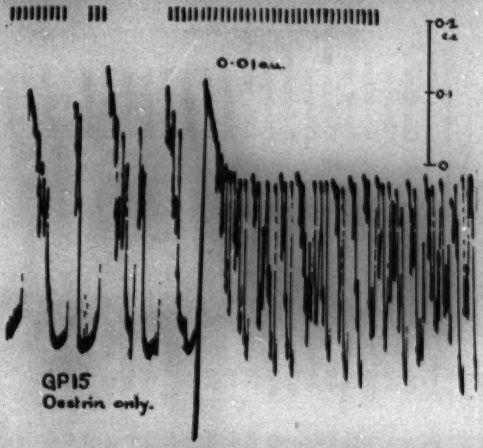

Fig. 4. GP 15. Animal treated with œstrin only. Response of the circular muscle of the uterus *in vivo* to the injection of 0·01 unit of oxytocin intravenously. Time intervals, 1 min.

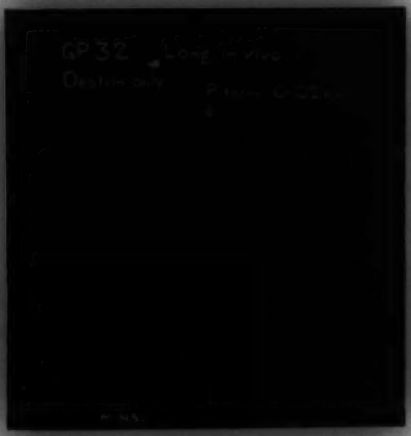

Fig. 5. GP 32. Animal treated with œstrin only. Response of the longitudinal muscle of the uterus *in vivo* to the intravenous injection of 0·02 unit of oxytocin. Time intervals, 1 min.

TABLE I. Summarizing all findings except those in which the longitudinal muscle was examined *in vivo*

Animal no. GP	Oestrin injection: Days	Oestrin injection: Total amount mg.	Progestin: Days	Progestin: Total amount	Reactivity to oxytocin: *In vitro*	Reactivity to oxytocin: *In vivo*	Spontaneous activity: *In vitro*	Spontaneous activity: *In vivo*	Remarks: Size of uterus	Remarks: Smear
2	3	0·06	—	—	—	—	—	8·0	Fairly large	Dioestrous
6	3	0·06	—	—	—	0·004	—	5·0	Small	Dioestrous
7	3	0·06	—	—	<0·005	>0·004	3·5; —	2·0	Small	Pro-oestrous
9	3	0·06	—	—	0·002	>0·01	1·5; 2·0	5·0	Smaller than medium	Pro-oestrous
14	3	0·06	—	—	0·001	>0·01	0·1; 0·5	4·0	Medium small	Early pro-oestrous
3	8	0·16	—	—	—	>0·004	—	5·0	Medium	Dioestrous
4	7	0·14	—	—	—	—	—	3·5	Small	Not recorded
15	7	0·14	—	—	0·001	<0·01>0·003	0·4; 0·7	10·0	Large	Pro-oestrous
17	7	0·14	—	—	0·002	0·003	1·0; 1·8	6·0	Moderate	Early pro-oestrous
23	7	0·14	—	—	0·001	0·003	0·1; 0·2	7·5	Medium	Dioestrous
10	3	0·06	3	6 c.c. own	0·002	—	2·0; 2·5	7·0	Moderately large	Dioestrous
5	7	0·14	3	6 c.c. own	0·0005	—	1·5; 1·5	—	Large	Dioestrous
8	7	0·14	3	6 c.c. own	0·001	<0·02>0·003	0·8; 0·8	7·0	Moderately large	Dioestrous
11	7	0·14	3	6 R.U. Organon	0·0005	<0·003>0·001	1·0; 2·0	4×2=8	Large, only half used	Dioestrous
12	7	0·14	3	6 R.U. Organon	0·002	<0·01>0·003	nil	4·0	Large	Dioestrous
13	7	0·14	3	6 R.U. Organon	0·002	<0·003>0·001	0·5; 3·0	2×2=4	Large, half used	Dioestrous
16	7	0·14	3	6 R.U. Organon	0·002	<0·02>0·003	0·1; 0·3	4·0	Moderately large	Dioestrous
18	7	0·14	3	6 mg. progesterone	0·001	>0·01	0·2; 0·6	3·5	Medium	Dioestrous
22	7	0·14	3	6 mg. progesterone	0·002	>0·02	0·7; 1·5	2·5	Largish	Dioestrous
				Gonadotropic hormone						
20	7	0·14	4	16 mg. M2	0·002	>0·003	0·2; 0·4	9·0	Medium	Pro-oestrous
21	7	0·14	4	17 mg. M2	0·002	>0·01	0·7; 1·0	8·0	Moderate	Not recorded
19	7	0·14	3	30 mg. M2	0·0005	>0·003	0·2; 0·4	10·0	Medium	Dioestrous

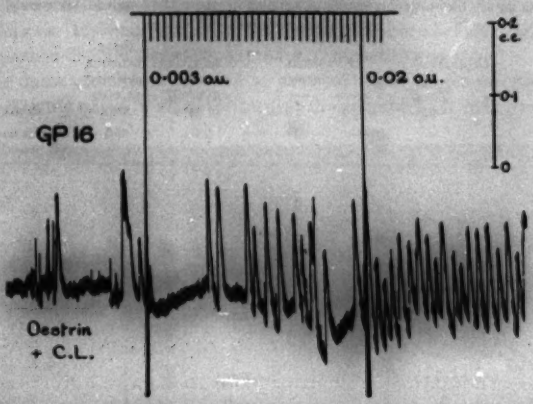

Fig. 6. GP 16. Animal treated with oestrin and corpus luteum extract. Response of the circular muscle of the uterus *in vivo* to 0·02 unit of oxytocin but not to 0·003 unit intravenously. Time intervals, 1 min.

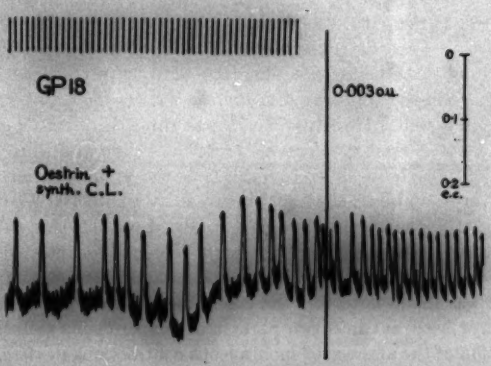

Fig. 7. GP 18. Animal treated with oestrin and progesterone. Absence of any response of circular muscle to intravenous injection of 0·003 unit of oxytocin. Notice the rhythmic contractions. Time intervals, 1 min.

TABLE II Showing the results in animals in which the longitudinal muscle movements were recorded *in vivo*

Animal no. GP	Oestrin		Progestin (Proluton)		Reactivity to oxytocin *in vivo*	Spontaneous activity *in vivo* mm.	Remarks
	Days	Total mg.	Days	Total			
34	—	—	—	—	0·01	0·5	Uterus small. Smear dioestrous
35	—	—	—	—	0·01	9·0	Uterus medium. Smear dioestrous
32	7	0·14	—	—	<0·02; >0·003	5·0	Uterus medium
33	7	0·14	—	—	0·02	5·0	—
36	7	0·14	3	6 R.U.	—	9·2	Uterus small. Smear dioestrous
37	7	0·14	3	6 R.U.	—	10·0	Uterus medium. Smear dioestrous

(*b*) *In vitro*

Certain features of the records obtained *in vitro* are worthy of comment. In the first place the base line was invariably level; the muscle always relaxed to its original length after the changes of solution following contractions produced by oxytocin. Further the amplitude of the spontaneous rhythmic contractions was very small in the majority of the experiments (see Table I). Moreover a change of solution (after addition of oxytocin to the bath) was not followed by an increase, and often was followed by a decrease, in the height of the spontaneous contractions as compared with the period preceding the addition of the drug; a typical series of responses is illustrated in Fig. 8.

Since it is well known that the uterus from the œstrous guinea-pig exhibits very marked spontaneous activity *in vitro* and since a similar high activity has been shown to result from the administration of œstrin to the ovariectomized animal [Marrian & Newton, 1933] our own results needed further investigation. It appeared possible that the absence of any marked spontaneous activity might be due to the interval of 3 days elapsing between the last injection of œstrone and the time of experiment. But this was found not to be the case. The fact that the uterine horns had always been cut into two parts in our experiments seemed a likely explanation of the absence of spontaneous contractions *in vitro*. A comparison was therefore made between the rhythmic activity of the whole horn and that of the half-horns removed from animals treated with œstrin over 7 days, the experiment being performed 3 days after the last injection of œstrin. It was found that the whole horn gave, as was expected, very marked contractions. On the other hand, the half-horns

from the same animals showed, as in the previous experiments, small contractions. It would thus seem that cutting the horn in half results in a very marked diminution of its rhythmic contractility *in vitro*.

The doses of oxytocin needed to produce motor effects *in vitro* were comparatively small in all experiments; thus no uterus failed to react to

Fig. 8. Illustrating the reactivity to oxytocin and the spontaneous rhythmic activity of the uterine half-horns suspended in oxygenated Ringer-Locke solution. The solution was changed after every addition of drug. Time intervals, 1 min.

0·005 unit per 100 c.c. and in the majority of experiments 0·002 unit was effective. In three cases a response was obtained with 0·0005 unit per 100 c.c. There was no appreciable difference between the spontaneous rhythmic activity exhibited *in vitro* by the uteri of groups 2, 3, and 4 (i.e. guinea-pigs treated with œstrin, with œstrin plus progestin, and with

œstrin plus gonadotropic hormone); and the reactivity to oxytocin *in vitro* was of the same order in these three groups.

A remarkable feature of the reaction to oxytocin is the marked difference between the concentrations of the drug necessary to cause contraction *in vitro* and *in vivo*. The concentrations *in vivo* (see Table III) have been calculated on the assumption that the blood volume of the guinea-pigs used was 20 c.c. The third column of Table III has been

TABLE III. Comparison of reactivity *in vitro* and *in vivo*. The reactivity has been expressed as the effective dose of oxytocin per 100 c.c. of Ringer-Locke solution or per 100 c.c. blood.

GP	Reactivity		Ratio of concentration
	In vitro	*In vivo*	*in vitro*/*in vivo*
14	0·001	>0·05	>50
9	0·002	>0·05	>25
7	<0·005	>0·02	>4
15	0·001	<0·05>0·015	15 to 50
23	0·001	0·015	15 or less
17	0·002	0·015	7·5 or less
18	0·001	>0·05	>50
22	0·002	>0·1	>50
5	0·0005	<0·1>0·015	30 to 200
8	0·001	<0·1>0·015	15 to 100
16	0·002	<0·1>0·015	7·5 to 50
11	0·0005	<0·015>0·005	10 to 30
12	0·002	<0·05>0·015	7·5 to 25
13	0·002	<0·015>0·005	2·5 to 7·5
19	0·0005	>0·015	>30
21	0·002	>0·05	>25
20	0·002	>0·015	>7·5

obtained by dividing the effective concentrations of oxytocin in the blood (i.e. oxytocic units per 100 c.c. of blood) by the minimum effective dose in 100 c.c. Ringer-Locke. It will be seen that in more than half the experiments the concentration necessary to cause contraction *in vivo* is more than 15 times the effective concentration *in vitro*. In three cases the discrepancy is greater than 50 times.

Vaginal smears were taken from all animals on the day of the experiment. In no case was a full œstrous smear obtained, although some of the animals showed various stages of pro-œstrous (see Table I). All the smears from animals injected with progestin in addition to œstrin were, however, completely dicestrous, i.e. they contained epithelial cells and leucocytes.

DISCUSSION

Since this investigation was carried out essentially to determine the possible action of the corpus luteum in the maintenance of pregnancy in an animal other than the rabbit, the results obtained with progestin will

be examined first. There seems little room for doubt that, under the experimental conditions described, progestin has no appreciable inhibitory action either on the reactivity to oxytocin (i.e. it produces no desensitization) or on the spontaneous rhythmic activity of the uterine muscle of the guinea-pig. This statement holds both for the investigations carried out *in vitro* and *in vivo*. Before we can apply these results to the condition of the animal during pregnancy it is necessary to determine whether the doses of hormones used are within physiological limits. In so far as œstrin is concerned it is obvious that the doses used were not equal to those normally involved in the production of œstrus, since a full vaginal cornification was never induced. Hence, although we have no information as to the amount of this hormone produced during pregnancy, we can at least say that the quantities used in these experiments were well within the known physiological limits. There is unfortunately no information as to what constitutes a physiological dose of progestin in the guinea-pig. The amounts we used would have been more than sufficient to produce the known actions of progestin in a rabbit weighing eight times more than some of the guinea-pigs used. In the mature ovariectomized rabbit treated with œstrin the subsequent administration of 0·75 mg. of progesterone over 4 days' results in a progestational proliferation of the endometrium similar to that seen at the height of pseudopregnancy; the same dose of progesterone also produces desensitization of the uterine muscle *in vitro* and *in vivo*, and inhibition of the spontaneous activity of the muscle observed *in vivo* [Robson, 1936]. Hence in the two animals injected with pure progesterone, some eight times the amount fully effective in the mature rabbit was given, and allowing for the difference in the body weight the dose in the guinea-pig was actually more than 50 times that necessary to cause proliferation of the endometrium and desensitization and inhibition of motility in the rabbit. Direct evidence that the doses of progestin used were, at least to some extent, effective in the guinea-pig is shown by the fact that in none of the animals treated with this hormone in addition to œstrin were œstrous changes produced in the vaginal smear—an inhibitory action of progestin similar to that recorded by de Fremery *et al.* [1934] in the mouse.

These results, therefore, suggest that progestin does not, in the guinea-pig, cause any inhibitory effect on the uterine activity similar to that described in the rabbit, and that any action of the hormone in the maintenance of pregnancy is not exerted on the reactivity and motility of the uterine muscle.

Furthermore, the results do not support the view that gonadotropic hormones may in the guinea-pig play a part in the control of uterine activity by a direct action on the muscle, since there is no evidence of any action on the uterine reactivity or motility with the hormone obtained from pregnancy urine. The doses of gonadotropic hormone used were greatly in excess of those necessary to produce quiescence of the uterus in the rabbit [Reynolds, 1932].

The experiments have revealed a discrepancy between the effective doses of oxytocin *in vitro* and *in vivo*. It is to be noted that the low reactivity *in vivo* applies both to the longitudinal and circular muscle. In the rat and in the rabbit, previous observers [Knaus & Clark, 1925; Robson, 1935, 1936] have obtained substantial agreement between the concentrations of drug effective on the uterus in the intact animal and on the uterus suspended in physiological solution. The cause of the discrepancy in the guinea-pig must remain at present unexplained; it may possibly be due to the fact that the reactivity to oxytocin of the guinea-pig uterus *in vitro* is usually higher than that of the uteri of other animals, e.g. the rabbit and the mouse, at similar stages of the sex cycle.

The results obtained from the ovariectomized untreated animals (group 1) require further comment. It was expected that in these animals the uterus would be atrophied and show only small rhythmic contractions, and such was the case in GP 34. In GP 35, on the other hand, the uterus on the tenth day after ovariectomy was still moderately large and exhibited marked contractions similar to those observed in the œstrin-treated animals. There is evidence, however, that œstrin may be produced in sites other than the gonads; Zondek [1934] has shown for example that quite appreciable quantities of œstrin are still excreted in the urine of non-pregnant castrated mares. Moreover, quite large quantities of œstrin have been extracted from certain organs, especially the suprarenals [Callow & Parkes, 1936]. It appears possible, therefore, that the uterus in the ovariectomized guinea-pig may be subjected to the influence of an œstrogenic substance derived from extra-gonadic sources.

Summary

Methods are described for recording the movements of the circular and longitudinal muscle of the uterus *in vivo*. One method, using an optical system, gives direct records with a high degree of magnification on a smoked drum.

Ovariectomized guinea-pigs were injected with œstrin and then with progestin or with gonadotropic hormones. The reactivity to oxytocin and

the spontaneous rhythmic activity of the uterus were then measured in the animal *in vivo*, and also on isolated strips.

One entire horn was used for *in vivo* experiments, while the other horn was cut into two portions for duplicate *in vitro* determinations.

Neither progestin nor gonadotropic hormones produced any desensitization of the reactivity to oxytocin or inhibition of the motility of the uterus, *in vitro* or *in vivo*.

The reactivity to oxytocin *in vitro* was high in all cases. The reactivity to oxytocin *in vivo* was smaller, and frequently much smaller than *in vitro*.

The spontaneous rhythmic activity *in vitro* was small; this was shown to be due to the halving of the horns.

The relation of the findings to the mechanism involved in the maintenance of pregnancy is discussed.

The expenses of this research have been defrayed by grants made by the Medical Research Council. We should like to thank Prof. A. J. Clark for valuable suggestions.

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612.817.1

EXPERIMENTS ON THE MECHANISM OF STRYCHNINE "CURARIZATION"

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(*Received* 30 *July* 1936)

It is well known that "curarization" of a nerve-muscle preparation can be obtained by other drugs than curare. Such a preparation is said to be "curarized" when the muscle can no longer be excited from its motor nerve, while the nerve itself and the muscle itself still maintain their normal excitability. Besides curare itself many other substances like the quaternary ammonium salts, eserine, veratrine, strychnine, etc., are known to give a typical block of the neuromuscular junction. The special properties of the neuromuscular junction have been explained on two different hypotheses: (*a*) that they are due to an intermediate substance which might take the form either of a special anatomical structure [Keith Lucas, 1906 *a*, *b*] or of a chemical body responsible for the transmission of the impulse from nerve to muscle [Dale, Feldberg & Vogt, 1936]: (*b*) that the neuromuscular junction should not be considered as a separate substance, but merely as a physiological mechanism, i.e. the muscle must be so adjusted as to be able to receive the impulse from the nerve [L. & M. Lapicque, 1906, 1908; Monnier, 1934; and others]. According to (*b*) it is essential for the conduction of the impulse from nerve to muscle that the time factor of excitation (the "chronaxie" [Lapicque, 1909]) should be the same in both. If by any means the time factor of either nerve or muscle is altered, the passage of the impulse becomes impossible as soon as one of the two time factors becomes twice as large as the other. Lapicque claims to have shown experimentally that any drug which is able to curarize a nerve-muscle preparation also gives a change in either muscular or nervous chronaxie, and according to him it is this change in chronaxie which is responsible for the neuromuscular block.

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According to this theory there are different types of "curarization". Curare itself acts by increasing muscular chronaxie, eserine by decreasing muscular chronaxie, as does veratrine in certain concentrations. Strychnine, however, in strong solutions, curarizes by decreasing nervous chronaxie, leaving muscular chronaxie unaltered [M. Lapicque, 1907; L. & M. Lapicque, 1908]. From these different types of curarization Lapicque derives the strongest argument in favour of his theory. If a nerve-muscle preparation is treated with veratrine in appropriate concentration "curarization" develops through decrease in muscle chronaxie, if the same preparation is also treated with strychnine nervous chronaxie also decreases and the two chronaxies may become equal again. According to the theory this should remove the "curarization". L. & M. Lapicque [1912] confirmed this by experiment, though Rushton [1933] was unable to obtain the result.

Lapicque's theory has met with many criticisms in recent years. Most of these are concerned with the question whether or not the chronaxies of normal motor nerve and its muscle are equal. Some criticisms have been offered to Lapicque's pharmacological experiments. Grundfest [1932] in experiments on single fibre preparations was unable to obtain the effect of curare on muscle chronaxie. Bouman [1932 *b*] showed that, after degeneration of nerve endings, eserine no longer changes muscular chronaxie. It cannot be denied, however, that Lapicque's crucial experiment, if correct, on the antagonism of the veratrine and strychnine intoxications is strong evidence in favour of his theory. There is another possible argument in favour of Lapicque's theory which has not hitherto been stressed: it is that, so far as the author is aware, "curarization", together with equality of nervous and muscular chronaxie, has never yet been described. In view of its importance as a fundamental point in the evidence for the theory of isochronism, a closer analysis of the strychnine "curarization" was undertaken.

Method. Gastrocnemius sciatic preparations of Hungarian frogs (*Rana esculenta*) were used throughout at room temperature (16–22° C.). All nerves were soaked in Ringer's fluid for at least 2 hours before commencing observations. Complete intensity duration (*E–RC*) curves were taken in each experiment. The results were plotted to see whether they fitted the theoretical equation [Hill, 1936 *a*, *b*]. The relatively few experiments which did not fit the equation were rejected. From the curves Hill's time constant of excitation k was measured. As Hill has shown, if the theoretical equation is obeyed, this time factor has a constant

relation to chronaxie (chronaxie = 0·693 *k*). Its use, however, has the advantage that the whole intensity duration curve is required for its measurement, thereby affording a check on the accuracy of the measurements, which—in several of the experiments to be described here—was found to be very necessary.

The impulse passing along the nerve was detected either by its action current, or by the response of the attached muscle. Unless stated otherwise, repetitive stimulation was used at a frequency of 20 and sometimes 10 per sec. Stimulating and lead-off electrodes on the nerve were calomel half-cells; in experiments on direct stimulation of the muscle silver-wire electrodes were employed.

Experiments with semi-maximal action current response. As Renquist & Parvianen [1931] have pointed out, certain difficulties may arise in obtaining complete strength-duration curves when using minimal muscle response as index of nerve excitation: these difficulties do not exist when the action current of the nerve itself is employed. Monophasic action currents were led off and integrated on a sensitive galvanometer [Hill, 1934, 1936 *b*; Scott, 1934]. It is best not to use minimal response [Hill, 1936 *b*] but a constant fraction of maximal. In the present experiments a semi-maximal action current was taken. The intensity-duration (*E–RC*) curve was determined before and after strychnine intoxication. A solution of 0·3 p.c. strychnine chloride in Ringer's fluid was used in all experiments, and the nerves were soaked in it for times up to 2 hours. Control experiments were performed on the opposite nerves of the same frogs, soaked in Ringer's fluid without strychnine for the same time. Table I gives some of the results. With the technique employed, no influence of strychnine on the time factor of excitation can be seen.

Experiments with minimal muscle response. The foregoing experiments are in complete opposition to Lapicque's on the effect of strychnine on nerve chronaxie [L. & M. Lapicque, 1908, 1913]. A series of experiments, therefore, was performed using minimal muscle response as index of nerve excitation. The muscle was attached to a very light spring. With repetitive stimulation a minimal muscle response gave a quite easily noticeable vibration of the spring. In some experiments the contractions were observed directly. When the entire nerve is soaked in the strychnine solution "curarization" develops and muscle response can no longer be observed. Only the part, therefore, of the nerve on which the electrodes were to be placed was soaked in the solution: according to L. & M. Lapicque [1908, 1913] this is sufficient to give a change in nerve chronaxie. The

TABLE I. The effect of strychnine on the time-factor k of nerve excitation; semi-maximal action current as index of constant response.

Exp. no.	k before soaking msec.	Soaking time (hours): Strychnine 0·3 p.c.	Soaking time (hours): Ringer's solution	k after soaking msec.
1	0·55	1½	—	0·54
2	0·57	1½	—	0·55
3	0·41	1½	—	0·52
4	0·33	2	—	0·40
5	0·51	1½	—	0·49
6	0·55	1	—	0·45
7	0·48	1½	—	0·51
	Mean = 0·49			Mean = 0·49
		Controls		
8	0·50	—	1½	0·49
9	0·65	—	2	0·69
10	0·52	—	2	0·60
11	0·60	—	1½	0·68
	Mean = 0·57			Mean = 0·61

soaking was done in a special paraffin-wax moist chamber, care being taken that the strychnine solution did not reach the neuromuscular junctions. The nerves were usually soaked for about 1 hour, but times up to 2 hours were occasionally employed. Control experiments were again performed on the opposite preparations of the same frogs similarly soaked in Ringer's solution without strychnine. Troubles of the type described by Renquist & Parvianen were easily eliminated, as the data of each strength-duration curve were only considered if they obeyed the theoretical relation. Table II gives some results. With the technique employed strychnine evidently decreases the time factor of excitation. This agrees completely with Lapicque's experimental results.

TABLE II. The effect of strychnine on the time-factor k of nerve excitation: minimal muscle response as index.

Exp. no.	k before soaking msec.	Soaking time (hours): Strychnine 0·3 p.c.	Soaking time (hours): Ringer's solution	k after soaking msec.
1	0·85	1½	—	0·59
2	0·85	3	—	0·47
3	0·80	1½	—	0·48
4	0·56	1	—	0·40
5	0·74	1	—	0·51
6	0·74	2	—	0·25
	Mean = 0·76			Mean = 0·45
7	0·89	—	1½	0·87
8	0·70	—	2	0·63
9	0·56	—	1	0·58
10	0·71	—	2½	0·66
	Mean = 0·71			Mean = 0·68

Experiments with semi-maximal muscular contraction. The unexpected disagreement between the results of the two foregoing series suggests that the way in which the nerve impulse is detected plays an important role. There are two differences between the two series of experiments: (i) in one series the nerve impulse was observed directly, in the other the muscle response, and (ii) in one series the response was semi-maximal, in the other minimal. It was necessary to test which of the two was responsible for the disagreement. Experiments with minimal nerve response often give rise to double curves [Hill, 1936 *b*] and the result of strychnine experiments was not always clear-cut, though it seemed as if one of the curves showed a change after strychnine intoxication. It was better, therefore, to investigate the strychnine effect with semi-maximal muscle response as index. Such experiments met with considerable difficulties owing to fatigue, especially at the neuromuscular junction. The maximum contraction height was determined at the beginning and at the end of each intensity-duration curve before and after intoxication. Experiments showing more than 10 p.c. variation in maximal contraction height were rejected. Moreover, all the curves used had to fit Hill's equation. Isometric contractions were used throughout. The frequency of stimulation was 2 per sec. Only about 20 p.c. of the experiments fulfilled all the requirements. Table III gives some of the results. With semi-maximal muscle response as index no effect of strychnine on the time factor of nerve excitation is found.

Table III. The effect of strychnine on the time factor *k* of nerve excitation: semi-maximal muscle response as index.

Exp. no.	*k* before soaking msec.	Soaking time (hours) Strychnine 0·3 p.c.	*k* after soaking msec.
1	0·55	1¼	0·49
2	0·53	1½	0·59
3	0·74	1½	0·68
4	0·46	1	0·44
	Mean = 0·56		Mean = 0·55

Discussion. From the above experiments it appears that the size of the constant response taken as index of excitation determines whether or not strychnine will change the time factor of nerve excitation. Under these circumstances it is hard to attach to this change any real significance in connexion with the failure of neuromuscular transmission. One possible explanation of the strychnine mechanism was suggested. Suppose that strychnine prevents excitation or conduction in the nerve

fibres with a long k more easily than in fibres with a short k. Lapicque & Legendre [1913] (see also Gasser [1935]) have shown that the fibres with the large k are usually the thinner, while Peugnet & Coppée [1936] have shown that very thin fibres are easily blocked by strychnine in the concentration used in the experiments described here. Whether this explanation really held could be tested as follows. The effect of strychnine was found only while using minimal muscle response as index of successful nerve excitation. If it is the slower fibres which are relatively more easily intoxicated by strychnine, and if it is these whose elimination affects the form of the strength-duration curve, then they must have a lower threshold than the faster ones, and when semi-maximal response is used their absence is more or less masked by the many faster fibres which are less sensitive to strychnine. In that case, as observed, the effect of strychnine would become less marked with a larger response as index of constant excitation. If this were true, however, we should find that the factor k determined with minimal muscle response was greater than the factor k determined with semi-maximal response.

Table IV gives some results of experiments on this point. In all of them the series of readings with semi-maximal response lay between two series with minimal response. The same precautions were taken as in

TABLE IV. The time-constant k of nerve excitation determined with minimal and with semi-maximal muscle response.

Exp. no.	k with minimal response msec.	k with semi-maximal response msec.	k with minimal response msec.
1	0·62	0·62	0·62
2	0·70	0·90	0·70
3	0·80	0·90	0·80
4	1·00	0·90	1·00
5	0·78	0·89	0·79
	Mean = 0·78	0·84	0·78

previous experiments that the maximal contraction height did not decrease appreciably during the determination of the strength-duration curve for semi-maximal contraction. It is clear from the table that if there is any difference between the values of k determined with minimal and with semi-maximal stimuli respectively, that difference is in the opposite direction to what was anticipated. The result agrees with previous observations [Bouman, 1932 *a*] in which a few points only of the curve were determined.

The explanation, therefore, suggested above, of the disagreement between results obtained with different sizes of index response, does not

hold. The question arose whether any after-effect of stimulation might be held responsible. Many theories of nerve excitation are based on the assumption that large quantities of potassium are liberated by the stimulus [see, for instance, Labes, 1932]. As far as the author is aware, no determination of this possible potassium liberation in medullated nerve has been made: the following experiments, therefore, were undertaken.

Experiments on potassium liberation in medullated nerve. The method was almost identical with that employed by Cowan [1934] in similar experiments on non-medullated nerve. A similar moist chamber was used. Eight sciatic nerves from eight different frogs were suspended on stimulating electrodes. The other eight nerves of the same frogs were suspended on similar electrodes in another chamber and used as controls. After being fixed in the chamber both sets were soaked in Ringer's solution. The solution was then carefully removed and thrown away. One of the sets of nerves was now stimulated with just maximal stimuli, 20 per sec. for 10 min., the current being reversed every 10 sec. Both sets of nerves were then soaked in a known amount of Ringer's solution for about 1 hour (the chambers being rocked regularly). The solution was then collected and its potassium content was determined by Dr J. F. Danielli, to whom I am greatly indebted. From Table V it is seen that a considerable amount of potassium is liberated during stimulation.

TABLE V. The potassium concentration developed in a nerve as a result of stimulation with 20 shocks per sec. in terms of the potassium concentration in normal Ringer.

Exp. no.	Stimulation time min.	Potassium concentration taking the potassium concentration of Ringer as a unit
1	10	25
2	10	15
3	15	35

Experiments on the influence of potassium on strychnine intoxication. From the experiments described the question arose whether or not the fairly large amounts of potassium liberated by a maximal stimulus might be responsible for the fact that no change in k was found with semi-maximal stimuli.

Experiments, therefore, were made in which the nerve was soaked in a 0·3 p.c. solution of strychnine hydrochloride in Ringer's solution containing more than the normal amount of potassium. Minimal muscle response was taken as index of excitation. Only the part of the nerve on which the electrodes were to be placed was soaked in the solution, care being taken that the solution did not reach the neuromuscular

junction. Control experiments were performed using Ringer with normal potassium concentration.

The soaking chamber consisted of a long groove in a paraffin block. Part of it was about 12 mm. deep and contained the muscle, the remaining part was about 22 mm. deep and contained the nerve. The two parts were separated by a low ridge over which the nerve passed. The solution was poured into the deeper part, the ridge preventing it from reaching the muscle.

In these experiments little effect on the factor k is found, even with the use of minimal muscle response as index. The controls show the usual decrease in k. With this result in mind two points must be considered.

(*a*) It is still hard to see how the potassium liberated in a series of semi-maximal stimuli, as used for the strength-duration curve with semi-maximal stimuli, can prevent the strychnine from decreasing the factor k.

TABLE VI. The effect of strychnine (0·3 p.c.) with excess potassium, on the factor k of nerve excitation: minimal muscle response. Experiments marked *a* and *b* were on the two nerve muscle preparations of the same frog.

Exp. no.	k before soaking msec.	Potassium in solution, as multiple of normal Ringer's solution	Soaking time hr.	k after soaking msec.	
1	1·00	10	1	—	0·90
2	0·73	7	1½	—	0·71
3 *a*	0·73	1	1	0·47	—
3 *b*	0·65	4	1	—	0·69
4 *a*	0·72	1	1	0·63	—
4 *b*	0·67	3	1	—	0·67
	Mean = 0·75			Mean = 0·55	0·74

It would seem more probable that most of the potassium which is liberated during the determination of the strength-duration curve before strychnine diffuses away during the time that the nerve is soaked in strychnine. It might, however, be the case that the potassium concentration, once present, leaves a permanent effect, so that even if the larger part of it diffuses away, the time factor of nerve excitation is no longer changed by strychnine. Experiments on this line will be given below.

(*b*) It has been shown that excess potassium abolishes the effect of strychnine on the time factor of nerve excitation. The question arises whether or not the mixed solution is still able to produce "curarization" in the usual way. Some experiments were performed, soaking the entire nerve muscle preparation in strychnine solutions containing four to ten times normal potassium. These experiments, however, did not give reliable results owing to the inexcitability of the muscle which develops

due to the excess of potassium [Dulière & Horton, 1929; Overton, 1904].

The following experiments have a bearing on both these points.

Experiments on the successive application of excess potassium and strychnine. To investigate the question whether the application of excess potassium would prevent strychnine from changing the time factor k, even if the major part of the potassium were removed before the strychnine was applied, the following experiment was performed.

Two nerve-muscle preparations were taken from the same frog. After previous soaking for 2 hours in Ringer's solution strength-duration curves were obtained, and k determined, using minimal muscle response as index. One of the nerves was then partially soaked in Ringer's solution containing excess potassium for 1 hour (only the part to be stimulated was soaked). Next the nerve was soaked in normal Ringer's solution for 1 hour to remove the major part of the potassium. Finally the same part of the nerve was soaked for 1 hour in 0·3 p.c. strychnine hydrochloride in normal Ringer's solution. Another strength-duration curve was then taken and the factor k determined. The other nerve-muscle preparation of the same frog was used as a control. It was soaked in exactly the same way, except that the fluid used in the hour of partial soaking was normal Ringer's solution without excess potassium.

It is clearly seen from Table VII that, while the control preparations showed the usual decrease in k which is obtained after strychnine alone (using minimal muscle response as index), the nerves which had previously been treated with excess potassium did not show this decrease.

It appears therefore that a nerve which has been exposed to excess potassium no longer has its k changed by strychnine, even if most of the potassium is washed out before the strychnine is applied. It is possible, therefore, to suppose that the excess potassium formed during the determination of a strength-duration curve with semi-maximal response is responsible for the failure of strychnine to change the factor k under these circumstances.

It was natural to enquire (i) whether the soaking times employed were really long enough to allow an appreciable amount of potassium to diffuse into the nerve, and (ii) whether in fact the major part of this potassium diffuses out again during subsequent soaking in normal Ringer's solution. This was tested by using another effect of potassium on nerve, i.e. the reversing effect on catelectrotonus. It has been shown [Chweitzer, 1935; Bouman, 1936] that potassium in fairly large concentration reverses the effect of a moderately strong catelectrotonus on

TABLE VII. The effect of previous soaking in Ringer's solution containing excess potassium, followed by removal of the major part of the excess potassium, on the change produced by strychnine in the factor k of nerve excitation. Minimal muscle response as index. Experiments marked *a* and *b* were on the two nerve muscle preparations of the same frog.

Exp. no.	k before K or strychnine msec.	Potassium in solution as multiple of normal Ringer's solution	k after treatment msec.	
1 *a*	0·70	4	0·65	—
1 *b*	0·81	1	—	0·80
2 *a*	0·81	4	0·81	—
2 *b*	0·85	1	—	0·45
3 *a*	0·75	4	0·80	—
3 *b*	0·65	1	—	0·35
4 *a*	0·85	4	0·88	—
4 *b*	1·00	1	—	0·65
5 *a*	0·78	4	0·78	—
5 *b*	0·74	1	—	0·55
6 *a*	0·80	4	0·75	—
6 *b*	0·75	1	—	0·50
7 *a*	0·60	4	0·61	—
7 *b*	0·61	1	—	0·52
8 *a*	0·80	4	0·80	—
8 *b*	0·83	1	—	0·53
9 *a*	0·70	4	0·78	—
9 *b*	0·73	1	—	0·51
	Mean = 0·73		Mean = 0·76	0·52

TABLE VIII. Columns A, B and C give the rise in threshold with catelectrotonus as percentage of the threshold before electrotonus: A in the nerve before treatment, B after 1 hour's soaking in Ringer's solution containing 10 times the normal potassium, C after the nerve had been soaked in normal Ringer's solution again for 1 hour

Exp. no.	Polarising current μA.	A	B	C
1	1	− 5	2·6	− 2
	2	−16	2·0	−28
	5	−26	− 2·6	−42
	10	−52	1·9	−45
2	1	−18	− 1·4	−10
	2	−18	2·8	−22
	5	−18	11·0	−30
	10	−27	38·0	−30
3	1	−18	− 1·3	−16
	2	−27	4·5	−24
	5	−44	31·0	−33
	10	−51	62·0	−37
4	1	−10	0	−14
	2	−22	0	−25
	5	−35	0·5	−39
	10	−42	4·4	−47
5	1	−15	0	−19
	2	−29	− 1·2	−33
	5	−49	− 1·2	−50
	10	−57	7·8	−54
	Mean =	−29	+ 8	−30

the excitability for constant currents, giving a decrease of excitability instead of an increase. In lower concentrations, or with shorter soaking times, the excess potassium may just abolish the effect of catelectrotonus on excitability. Table VIII gives the results of some experiments on this point. The threshold change by electrotonus is expressed as a percentage of the threshold without electrotonus. After 1 hour's soaking there is a clear-cut effect of potassium, and this effect almost completely disappears if the potassium treatment is followed by 1 hour's soaking in normal Ringer's solution.

It has been shown that successive treatment with potassium and strychnine gives no change in the time factor k of nerve excitation. The important question arises, does this treatment still give "curarization" when applied to the entire preparation, and if so how can this be explained on the basis of the theory of isochronism? To test this, complete nerve-muscle preparations were given the same treatment as previously was applied to the nerve, namely, (i) soaking for an hour in Ringer's solution containing four or more times the normal amount of potassium, followed by (ii) soaking for an hour in normal Ringer's solution, and then (iii) soaking in 0·3 p.c. strychnine hydrochloride in otherwise normal Ringer's solution. In all the experiments "curarization" resulted, nerve and muscle remained separately excitable, while transmission from nerve to muscle was completely abolished.

It is a well-known fact that the inexcitability of muscle which results from the application of excess potassium is easily reversible. It is important in these experiments to use oxygenated Ringer's solution throughout, owing to the increase in resting metabolism in potassium-soaked muscles [Solandt, 1936].

Can this "curarization" be explained on the basis of Lapicque's theory of heterochronism? We have seen that the excess-potassium-strychnine treatment gives no change in the time factor k of nerve excitation. According to Lapicque strychnine alone does not change the muscular chronaxie of muscle. The combination, however, of strychnine and potassium might change it. Direct measurement of the muscle chronaxie before curarization is difficult because direct stimulation is then usually a mixture of excitation of muscle fibres and of intramuscular nerve twigs. Experiments, therefore, were made as follows. A strength-duration curve was taken with indirect stimulation, using minimal muscle response as index, and the time factor k of nerve excitation determined. According to Lapicque's theory of isochronism the value of k observed for nerve is also that of muscle. Next the entire preparation was soaked for 1 hour in Ringer's solution containing excess potassium and

then in normal Ringer's solution for 1 hour. Finally the preparation was soaked in strychnine-Ringer's solution. "Curarization" has then developed, so that the time factor k of muscle can now be determined by direct stimulation. Table IX gives some of the results.

TABLE IX. The effect, on neuromuscular isochronism, of successive treatment with excess potassium (of which the major part is removed again) and with strychnine.

Exp. no.	k of nerve before treatment msec.	Potassium as multiple of normal	k of muscle after treatment msec.
1	0·80	4	0·80
2	0·75	4	0·85
3	0·87	4	0·82
4	0·55	4	0·55
5	0·65	4	0·65
6	1·10	4	0·80
7	0·75	7	1·45
8	0·62	7	1·10
	Mean = 0·76		Mean = 0·88

According to Lapicque a relation between the two chronaxies of 1 : 2 is necessary for curarization. We see, however, that this relation is never reached, and are forced to conclude that the "curarization" in question cannot be explained by a change in muscle chronaxie. We have seen that this treatment does not change nerve chronaxie, and are forced to the conclusion that successive treatment with excess potassium and strychnine gives rise to a "curarization" which is not accompanied by heterochronism. In view of the important place which strychnine intoxication took in Lapicque's arguments one must look for another explanation of the mechanism of "curarization". Peugnet & Coppée's [1936] experiments on the intoxication of very small nerve branches seem to point to intermuscular nerve twigs and possibly to Boeke's peri-terminal network [Boeke, 1921; Heringa, 1931]. Bouman's [1932 *b*] experiments on physostigmine "curarization" may have to be explained in the same way.

SUMMARY

Lapicque's conclusion that nerve chronaxie is decreased by strychnine is confirmed, using repetitive stimulation and determining the time-constant k from a whole strength-duration curve. If, however, the minimal muscle response as index of excitation is replaced by semi-maximal muscle response, or by semi-maximal nerve action current response, no change in the time factor of excitation is found.

This effect is not due to a greater sensitivity to strychnine of fibres with a greater time constant.

It is possible that excess potassium formed during the determination of the strength-duration curve is responsible. During similar stimulation potassium is in fact liberated.

If excess potassium is added to the strychnine solution no effect on the time factor of nerve excitation can be found, even when minimal muscle response is used as index. If a nerve is soaked in excess potassium, then in Ringer's solution and finally in strychnine, no change in the time factor of nerve excitation is observed. This treatment, however, gives normal "curarization". There is no evident change in the time factor of muscle excitation by such treatment. This type of "curarization", therefore, is not accompanied by neuromuscular heterochronism.

I am greatly indebted to Prof. A. V. Hill for the hospitality offered to me in his laboratory and for innumerable suggestions and constant advice, and to Mr J. L. Parkinson for much help.

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SYMPATHETIC VASO-DILATOR FIBRES IN THE HARE AND THE MONKEY COMPARED WITH OTHER SPECIES

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(Received 8 August 1936)

We have recently investigated the sympathetic innervation of the vessels in the different tissues of the dog; we found [1935] that among the fibres running in the abdominal sympathetic chain to the muscles of the hind-limbs there were many which were vaso-dilator in function, and which could be demonstrated without the use of ergotoxine. The alteration in vascular tone produced by the constant infusion of a dilute solution of adrenaline into a vein, although it did not greatly affect the blood pressure, was sufficient to change the response from a constriction to a dilatation of the muscle volume when a brief stimulation was applied to the sympathetic chain. The vaso-dilatation was found to be due to the liberation of acetylcholine since it was enhanced by eserine and abolished by atropine, and since acetylcholine was found in the venous effluent in perfusion experiments during sympathetic stimulation. In later work [1936] we investigated the sympathetic supply of the vessels of the dog's intestine and of the dog's skin; in neither of these were cholinergic vaso-dilator fibres present; after the injection of ergotoxine, vaso-dilator fibres were easily demonstrable in the splanchnic nerve, but they appeared to be adrenergic in function; in the skin no vaso-dilator sympathetic fibres were found at all except in the blood vessels of the ear.

The first indication of a difference in the sympathetic supply of different species was given by the observation of Dale [1906] that in the rabbit the pressor action of adrenaline is only diminished by ergotoxine, while in the cat it is usually reversed. Recently Rosenblueth, Schlossberg & Cannon [1935] have stated that there are no sympathetic vaso-dilator fibres

in the rabbit because, after giving ergotoxine, stimulation of the lumbar sympathetic chain does not cause a fall in the general blood pressure. Our own experiments [1935] showed that in the same circumstances a fall occurs in the cat, this fall being attended by an expansion of the muscle volume. The much greater and more easily demonstrated vaso-dilator effects in the dog indicate, however, that just as the cat differs from the rabbit, so the dog differs from the cat, and that in passing from one species to the other we go from the rabbit, in which sympathetic vaso-dilator fibres do not exist, to the cat in which they exist but are not demonstrable save by the use of ergotoxine, and then to the dog in which they can easily be demonstrated in several ways, and in which the vaso-dilator fibres to the muscles are entirely cholinergic.

The functional importance of a centrally controlled system of vaso-dilator fibres has not so far been recognized. Dale [1936] has expressed the view that the central nervous control of the blood vessels is a constrictor control by means of which blood can be cut off from any portion of the periphery. He has pointed out that the hormonal control which is exercised through the suprarenal glands and possibly also through the posterior lobe of the pituitary body is in the main also a constrictor control. He suggests that all important vaso-dilator effects are brought about by local vaso-dilator substances or by local axon reflexes.

By the evidence which we have obtained in the course of our work, we have been led to doubt whether this view can be accepted without modification. When the rabbit, the cat and the dog are compared, they are seen to have different powers of endurance. The rabbit is capable of running for a relatively short distance; it can dash to the nearest rabbit hole. The cat can probably exert itself for a longer time than the rabbit, but both animals are greatly inferior to the dog, which can run for miles without fatigue. When an animal's powers of endurance are subjected to strain, we may assume that its sympathetic system is not quiescent, but is in action; if now the effect of sympathetic activity is to reduce the blood supply to the muscles, it is clear that this activity will, by this effect, reduce the animal's powers of endurance and make it the more easily fatigued. If, on the other hand, the sympathetic system has a double function towards the muscular blood supply, not only to reduce it when the needs of the coronary circulation and of the brain are great, but also to increase it when muscular activity is prolonged, then we can understand that the dog, in which the two kinds of fibres are so easily demonstrable, has so much greater a capacity for prolonged muscular work than the rabbit or the cat.

We therefore formed the hypothesis that the sympathetic vaso-dilator fibres in the dog are in part responsible for the increased power of endurance which it possesses, and we have proceeded to test our view by examining other species. The hare seemed a specially suitable animal; its diet and habits are in many ways similar to those of the rabbit, but its powers of endurance are out of all proportion greater; like the dog it can run for several miles. We have therefore examined the hare and have compared it not only with the rabbit, as bred in captivity, but also with the wild rabbit.

In passing from the rabbit to the cat and then to the dog, we are not only passing to animals of greater muscular powers but also to animals higher in the scale of evolution. The greater prominence of sympathetic vaso-dilator fibres in the dog can therefore be explained as a part of the dog's more complete development, unconnected with its powers of endurance. To test this we have examined the monkey, which is still more highly developed but which has no ability to perform prolonged muscular work. The *Macacus rhesus* is easily fatigued.

Observations on hares

(*a*) *Methods*

Our observations have been made on wild hares on which the experiments were carried out a day or two after they were caught. We made plethysmograph records of the volume of one hindlimb after removing the skin. The hares were first anæsthetized with ether, after which "Pernocton" (sodium butyl-β-bromallylbarbiturate 10 p.c.) was given by intravenous injection. They were eviscerated, and the lumbar sympathetic chain was prepared for stimulation. In some experiments in which vaso-dilator substances were injected, ether was used as anæsthetic throughout and the vagi were cut.

(*b*) *Experimental results*

(i) *The effect of sympathetic stimulation.* The changes in the volume of the muscles of the hindlimb when the sympathetic chain is stimulated are illustrated in Fig. 1, which shows the effect of a stimulus applied for 1 sec. in (*a*) and for 30 sec. in (*b*). The short stimulus produced an initial constriction which, when the stimulus stopped, immediately gave place to a dilatation exceeding the constriction in extent. The dilatation appeared to be superimposed upon the constriction, for as the dilatation passed away, the phase of constriction reappeared. The long stimulus produced

a similar effect; while it was applied constriction occurred; when the stimulus ceased, the constriction was interrupted by a rapid dilatation, to be resumed as the dilatation passed away. The dilator phase of each stimulation was accompanied by a fall in the blood pressure.

Fig. 1. Hare anæsthetized with Pernocton. Upper tracing is the volume of the hindlimb after removal of the skin; downstroke indicates constriction; lower tracing is the blood pressure. Brief stimulation (*a*) of the lumbar sympathetic chain caused initial constriction giving place to large dilatation. Prolonged stimulation (*b*) gave constriction followed by dilatation.

In the dog vaso-dilator effects produced in this way are due to the liberation of acetylcholine; they are readily seen during the intravenous infusion of adrenaline or after the injection of eserine, and are abolished by atropine. In the hare the infusion of adrenaline or the injection of eserine did not magnify the dilator phase of the response, and it remained unaffected by the injection of atropine (see Fig. 2).

Therefore we concluded that the dilatation was not due to the liberation of acetylcholine, and was likely to be due to the liberation of an adrenaline-like transmitter.

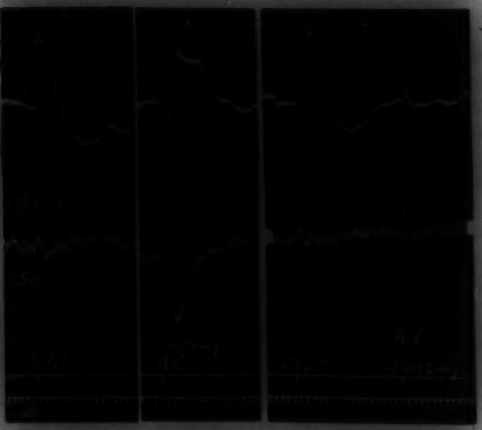

Fig. 2. Hare. Records as in Fig. 1. (*a*) The mainly dilator effect of stimulation; (*b*) the effect of acetylcholine. Between (*b*) and (*c*) 5 mg. atropine were injected. In (*c*) the effect of stimulation remains unaltered while that of acetylcholine is abolished.

Fig. 3. Hare. (*a*) and (*b*) Sympathetic stimulation. Ergotoxine (0·75 mg.) was then given. (*c*) and (*d*) show that the dilatation is abolished and the constriction reduced.

The effect of the injection of ergotoxine on the response was surprising; we expected to observe an uncomplicated vaso-dilatation, and found instead the reverse; ergotoxine abolished the dilatation, but not the constriction. This is illustrated in Fig. 3. Soon after the injection of 0·75 mg., the constrictor effect was somewhat reduced, but the dilatation was absent; a little later, in response to a further stimulus the dilator phase was again seen, though it was very small. The effect of the relatively small dose of ergotoxine was already declining, but it was difficult to make comparable observations after larger doses since in the hare they cause a severe fall of blood pressure.

Fig. 4. Hare. (*a*) Sympathetic stimulation; (*b*) the partial disappearance of the dilatation after 5 mg. cocaine; (*c*) the complete disappearance of dilatation after 10 mg. cocaine; (*d*) the simple constrictor response of the ear vessels to stimulation of the cervical sympathetic; (*e*) its prolongation after 9 mg. cocaine.

Cocaine has been used by Rosenblueth & Schlossberg [1931] and by Cannon & Rosenblueth [1933, 1935] to increase the effect of sympathetic stimulation in the body; its action in increasing the pressor response to adrenaline is well known, and Cannon & Rosenblueth find that it also increases the depressor response obtained after previous injection of ergotoxine.

We therefore tested the effect of cocaine on the response to stimulation in the hare. Here again the result was different from expectation; after the injection of 5 mg. cocaine (Fig. 4*b*) the dilatation was diminished, and after the injection of 10 mg. it was abolished. The constriction was not increased in amount, but it persisted for a longer time. This pro-

longation was clearly seen when plethysmograph records of the ear volume were taken (Fig. 4*d*, *e*). In the ear of the hare, stimulation of the cervical sympathetic produces simple constriction, which after cocaine lasts longer than before.

Alcock & Daly [1936] have recently described the augmentation of the effect of tyramine on the pulmonary arterioles by previous injection of both ergotoxine and cocaine. We took the opportunity to see whether the combination of these substances had any other effect on the response to sympathetic stimulation than either alone. We found that the response obtained after giving ergotoxine was not modified by later injection of cocaine and, similarly, the response obtained after cocaine was unaffected by injecting ergotoxine.

(ii) *The effect of sympathetic stimulation in the rabbit.* So far as we are aware there are no records in the literature showing the effect of sympathetic stimulation on the muscles of the rabbit's limb; we have, therefore, made a series of experiments for comparison with the hare. We have used not only rabbits bred in captivity, but also wild rabbits. When we found that the reactions of the hare were different from those of the tame rabbit, we felt that we ought to ascertain whether the reactions of the hare were due to the very different life it lived in its wild state. We therefore examined a series of wild rabbits caught in the same neighbourhood as the hares. The reactions in wild rabbits were the same as those in tame rabbits; we observed no differences. The animals used were similar in weight to the hares and the amount of muscle enclosed in the plethysmograph was also similar. Nevertheless the effects were much smaller, and were almost always a simple constrictor response with no accompanying dilator component; they resembled that in the ear of the hare both in shape and in size (Fig. 4*d*), the volume change in the rabbit's leg being no greater than that in the hare's ear. In one experiment, from which the record in Fig. 5 is taken, the form of the volume change was similar to that seen in the hare; the initial constriction quickly disappeared, and then was succeeded by

Fig. 5. Tame rabbit. (*a*) Sympathetic stimulation on the volume of the hindlimb—usually the response is a simple constriction, but in this experiment the form has some resemblance to that seen in the hare; (*b*) after 1·2 mg. ergotoxine; stimulation has no effect.

a smaller constriction slowly coming on, and slowly passing off. In this rabbit the response could be interpreted as a constriction with a superimposed phase of dilatation, but, as already pointed out, it was an effect not seen in other rabbits.

The second part of Fig. 5 illustrates that after the injection of ergotoxine the stimulation of the sympathetic chain had no effect. The injection of cocaine increased both the pressor response to adrenaline, and also the constrictor response to stimulation, the increase being not merely in the duration, but also in the size, of the response (Fig. 6).

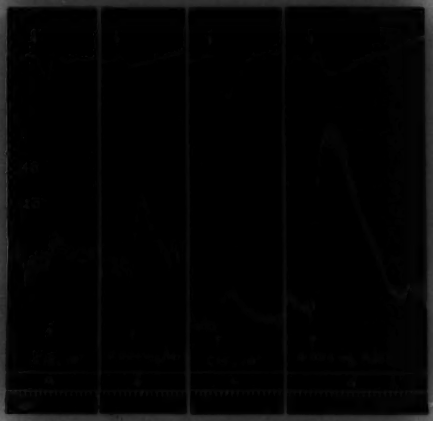

Fig. 6. Tame rabbit. (*a*) The constriction obtained when the sympathetic chain is stimulated; (*b*) that following the injection of 0·004 mg. adrenaline. 5 mg. cocaine was then injected. Both stimulation (*c*) and adrenaline (*d*) had a much increased effect.

(iii) *The effect of vaso-dilator substances.* Since the injection of ergotoxine and of cocaine abolished the dilatation seen in the hare when the sympathetic chain was stimulated, we examined the action of vaso-dilator substances. The dilator effect of acetylcholine is illustrated in Fig. 2 and also in Fig. 7. The response of the muscles is large like that of the muscles of the dog, and is abolished by atropine.

Small doses of adrenaline also have a large vaso-dilator action though this is not accompanied (Fig. 7) by a fall of blood pressure; there is indeed a brief rise of pressure, which, when the record is taken on a slow drum, looks as though it may be responsible for the increased volume of blood

in the muscles. On a faster drum, however (see Fig. 7*c*), the main dilatation is seen to occur as this rise of pressure disappears; such a dilatation ought to cause a fall in the blood pressure, and it is curious that there is none; presumably the effect of the muscle dilatation is compensated by constriction in the splanchnic area or in the skin.

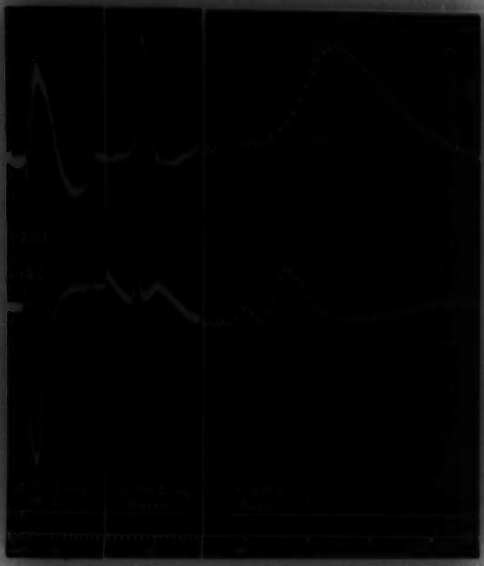

Fig. 7. Hare. (*a*) The dilator effect of acetylcholine (0·002 mg.) in the hindlimb muscles; (*b*) the dilator effect of adrenaline (0·002 mg.); (*c*) is the same as (*b*) but taken on a faster drum to show that the main dilatation occurs as the blood pressure falls.

Other vaso-dilator substances which were examined were adenosine, histamine and tyramine. The injection of 1 mg. adenosine produced a fall of blood pressure and vaso-dilatation (Fig. 8*c*). Histamine, which except in certain circumstances [Feldberg, 1927] has little or no vaso-dilator action in the rabbit, was found to have no depressor effect or vaso-dilator effect in the hare; we attribute the initial volume increase shown in Fig. 8*b* to the increase of blood pressure. Since the limb volume fell while the pressure remained high, we think that there was some vaso-constriction in the muscles.

The injection of a small dose of tyramine was given because tyramine has some dilator action in the dog and also in the cat after the injection of cocaine [Burn & Tainter, 1931]. The tracing in Fig. 8*a* indicates that 0·1 mg. caused vaso-dilatation, though there was also a slight pressor effect. Since the increase in limb volume was much greater than that produced by 0·02 mg. histamine, and since the pressor effect of the tyra-

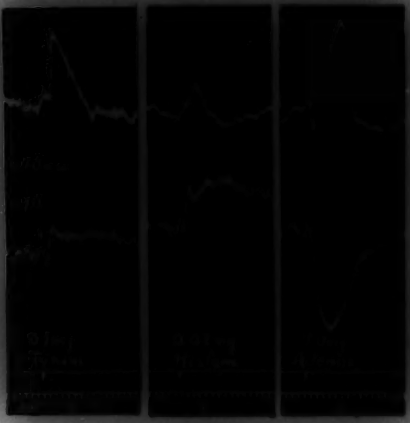

Fig. 8.

Fig. 9.

Fig. 8. Hare. (*a*) A slight dilator effect of 0·1 mg. tyramine; (*b*) no dilatation following 0·02 mg. histamine (see text); (*c*) a good dilator effect of 1 mg. adenosine.

Fig. 9. Wild rabbit. (*a*) Effect of 0·002 mg. adrenaline; (*b*) dilatation following 0·002 mg. acetylcholine; (*c*) a trace of dilatation following 0·4 mg. adenosine.

mine was much less than that due to the histamine, we think that the increase in volume caused by the tyramine was not a passive effect secondary to the blood-pressure change.

(iv) *Dilator effects in the rabbit.* We have examined the action of these substances in the rabbit, and have found that none of them has a dilator effect at all comparable in size with that in the hare. Both acetylcholine and adenosine have a depressor action, but the change in the volume of the muscle vessels which occurs simultaneously is very small. Fig. 9 is a record which shows that a small dose of adrenaline (0·002 mg.) had no depressor effect (Fig. 9*a*); the limb volume expansion was probably

passive. Larger doses were constrictor (see Fig. 6*b*). Acetylcholine was the only substance producing indisputable dilatation (Fig. 9*b*); after 0·4 mg. adenosine, the volume of the limb did not rise appreciably above the original level. Tyramine had no dilator effect in small doses, and the pressor effect of bigger doses was feeble compared with that in the hare.

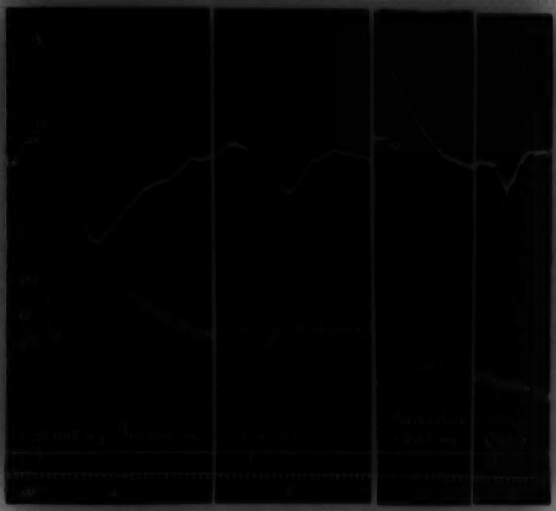

Fig. 10. Hare. (*a*) Initial dilatation succeeded by constriction when 0·005 mg. adrenaline was injected; (*b*) response to stimulation. Between (*b*) and (*c*) 0·75 mg. ergotoxine was given. (*c*) shows that the initial dilatation caused by adrenaline remains, though the later constriction is abolished. Between (*c*) and (*d*) 3·0 mg. ergotoxine injected. (*d*) shows that stimulation causes only constriction, the dilator component of the response being abolished.

(v) *The humoral transmitter in the hare.* We have no final evidence concerning the humoral transmitter causing vaso-dilatation in the hare, but certain observations may be made. The transmitter is unlikely to be acetylcholine for reasons already given. The next assumption would be that it is adrenaline, but two observations at least do not support this. We have shown that ergotoxine abolishes the dilatation caused by stimulation, but it does not alter the dilator effect of adrenaline. In Fig. 10 is shown for comparison the effect of 0·005 mg. adrenaline and also

of sympathetic stimulation before and after ergotoxine. In (*a*) the injection of adrenaline produced an initial expansion of muscle volume followed by constriction; in (*c*) the same injection given after 0·75 mg. ergotoxine produced a similar expansion, but the constriction was abolished. It is possible to regard the initial expansion of the muscle as a passive effect due to the rise of blood pressure, but since it also occurred after ergotoxine when the pressor effect was trifling, we think the expansion was a dilatation similar to that shown in Fig. 7. The dilatation produced in Fig. 10*b* by stimulation was abolished after ergotoxine, though even when 3 mg. was injected, some constrictor effect remained (*d*). The evidence therefore suggests that the dilatation produced by stimulation is not due to adrenaline. In support of this is the further observation that the initial dilator effect of a small dose of adrenaline was increased after the injection of cocaine; whereas, as shown in Fig. 4, the dilatation following stimulation was abolished by cocaine.

Another possibility is that adenosine is the humoral transmitter; we found, however, that the dilatation caused by this substance was unaffected by the injection of either ergotoxine or cocaine. As already described, small doses of tyramine caused a dilatation, which was also seen as the initial effect of bigger doses; this initial dilatation was again not affected by ergotoxine or cocaine. It was interesting to observe that, in the hare, cocaine did not reduce the pressor action of tyramine as it does in the cat.

Observations on monkeys

(*a*) *The effect of sympathetic stimulation*

We have made observations on a series of monkeys (*Macacus rhesus*) in the same way as on hares; in addition we have perfused the hindlimbs of the monkey with hæmoglobin-Ringer [Amberson & Höber, 1932]. When the lumbar sympathetic chain is stimulated in the monkey anæsthetized with Pernocton, the volume of the hindlimb diminishes. The constriction is about the same in the intact leg as it is after removal of the skin; in this respect the monkey differs from the dog, for, as we have shown [1936], the constriction in the dog's leg is very much less after removal of the skin. The vaso-constrictor effect is not reversed by ergotoxine as in the cat and the dog. Fig. 11 shows in (*a*) the vaso-constrictor response to brief stimulation of the chain; in (*b*) a constrictor effect produced by a small dose of adrenaline; in (*c*) a constrictor response to stimulation after 3 mg. ergotoxine; and in (*d*) a dilator response to a dose of adrenaline ten times as great as in (*b*). The administration of increasing

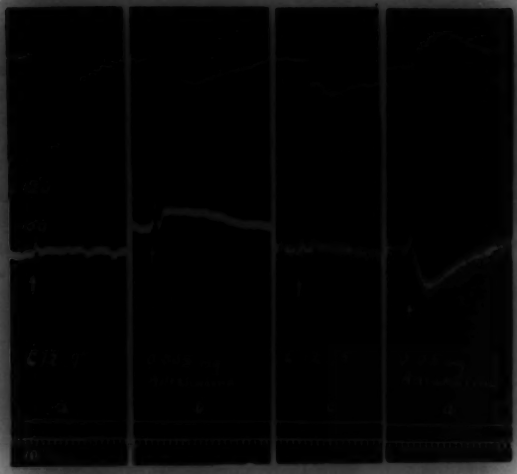

Fig. 11. Monkey, Pernocton anæsthesia. (*a*) The constrictor response of the skinned hind-limb to brief stimulation of the sympathetic chain; (*b*) the constriction produced by 0·005 mg. adrenaline. Between (*b*) and (*c*) 3 mg. ergotoxine were injected. (*c*) shows that the constrictor effect of stimulation still persists, although (*d*) the adrenaline effect has been reversed.

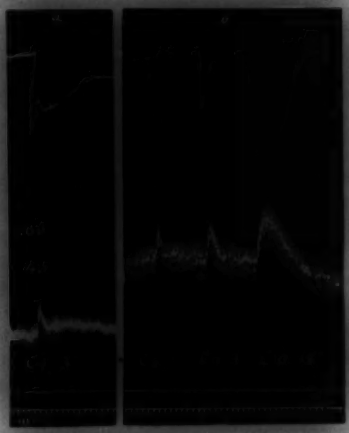

Fig. 12. Monkey. Pernocton anæsthesia. The constrictor response to stimulation seen in (*a*) is not converted to dilatation in (*b*) during the intravenous infusion of adrenaline. This conversion regularly takes place in the dog.

amounts of ergotoxine (we have given as much as 27 mg.) causes an increasing paralysis of constrictor fibres until stimulation of any strength or duration has no constrictor effect; but vaso-dilatation is not observed. On the other hand, the injection of adrenaline is followed by a fall of blood pressure and vaso-dilatation.

In the dog the infusion of adrenaline into a vein reverses the constrictor response in the muscles when the chain is stimulated; no such action was observed in the monkey, as shown in Fig. 12; during the infusion of adrenaline the vaso-constriction produced by a given stimulus was only diminished.

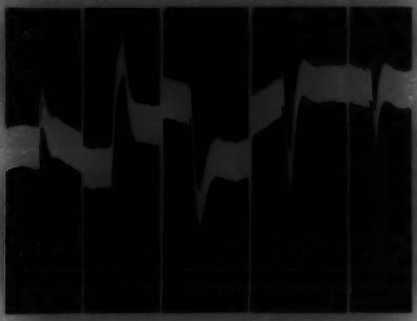

Fig. 13. Monkey. Perfusion of hindlimbs with haemoglobin-Ringer's solution, to which adrenaline was added to raise the tone. (*a*) Stimulation of the sympathetic chain for 3 sec.; (*b*) for 20 sec.; (*c*) the dilatation produced by intra-arterial injection of 0·02 mg. histamine; (*d*) the transient dilatation produced by 0·02 mg. acetylcholine; (*e*) the effect of 0·002 mg. acetylcholine.

When the vessels of the dog's hindlimbs are perfused with defibrinated blood containing adrenaline, the response to stimulation of the chain is almost always vaso-dilatation. We tested the effect of stimulation when the vessels of the monkey were perfused with haemoglobin-Ringer; the Ringer was re-oxygenated in the apparatus described by Euler & Heymans [1932]. The tone of the vessels was raised to a constant level by adding adrenaline (1 in 50,000) at a uniform rate to the venous reservoir. Stimulation of any duration was followed by vaso-constriction, as shown in Fig. 13; the effect of applying a stimulus for 20 sec. with the secondary coil at 10 cm. was observed to be similar to the effect of injecting 12·5γ adrenaline into the arterial cannula tied in the abdominal aorta.

(b) Action of vaso-dilator substances

The interesting observation was made that acetylcholine has very little depressor or vaso-dilator action in the monkey anæsthetized with Pernocton. In some animals a slight effect was seen as shown in Fig. 14, but in others doses up to 0·05 mg. had no effect even on the blood pressure. Adenosine had more action than acetylcholine, though the vaso-dilatation it produced was slight, but the only substance which exerted a good

Fig. 14. Monkey. Upper tracing, volume of skinned hindlimb; middle tracing, volume of intact hindlimb. (*a*) shows that acetylcholine has a very slight dilator effect; (*b*) shows a somewhat greater dilatation due to adenosine and a good dilatation due to histamine.

depressor and dilator effect was histamine (see Fig. 14). These observations were confirmed in the perfused limbs of the monkey, though a dose of 0·02 mg. acetylcholine injected into the arterial cannula then produced an appreciable though brief dilatation (Fig. 13*d*).

OBSERVATIONS ON GREYHOUNDS

According to our hypothesis the development of vaso-dilator fibres in the sympathetic system of the dog is connected with the dog's greater capacity for prolonged muscular effort. We have therefore investigated whether vaso-dilator fibres are present to a greater extent in greyhounds

than in dogs of other varieties. As already mentioned, the constriction which occurs in the muscles of the skinned leg when the lumbar sympathetic chain is stimulated is relatively small compared with that observed in the leg with the skin on; we thought that in the greyhound the vaso-dilator fibres might be so numerous that constriction would not be

Fig. 15. Greyhound. Pernocton anæsthesia. Stimulation of the sympathetic chain (*a*) caused vaso-constriction although the blood pressure was 200 mm.; (*b*) 0·005 mg. adrenaline; (*c*) after 1·5 mg. ergotoxine; the effects of stimulation and of adrenaline are both dilator and depressor.

obtained at all in the skinned limb, at least to stimuli of short duration. Experiment showed that this was not so; the result in Fig. 15 was specially clear because the blood pressure was as high as 200 mm. and gave a good opportunity for the observation of vaso-dilatation. Brief stimuli regularly produced constriction in the muscles; after the administration of ergotoxine, the stimuli produced vaso-dilatation. The difference between the dog and the monkey is seen at once when Figs. 11 and 15 are compared.

Discussion

The results given enable a comparison to be made between the rabbit, the hare, the cat, the dog and the monkey. Vaso-dilator fibres are present in the sympathetic supply of the muscles of the cat, the dog and the hare, but not in the supply of the rabbit or the monkey. In the cat they are demonstrated only by the administration of ergotoxine, and the vaso-dilator effects are small. In the dog they can be demonstrated by an intravenous infusion of adrenaline, or by the injection of eserine or ergotoxine; the vaso-dilator effects in the dog are large. In the hare the administration of drugs is not needed; vaso-dilatation is obtained in ordinary circumstances as a phase of the response to stimulation of the sympathetic chain, of which constriction is another phase; when the stimuli are brief the dilatation exceeds the constriction. For the dog there is a clear difference between the effects of short and long stimuli, the former—under certain conditions—causing immediate dilatation, the latter vaso-constriction. For the hare there is a similarity between long and short stimuli, the difference being quantitative rather than qualitative. The initial effect is always constrictor which continues while stimulation is applied and therefore becomes bigger the longer stimulation lasts. As soon as the stimulation stops, the dilatation appears, and the size of the dilatation is dependent on how far the vessels were constricted before.

The fibres in the cat are in the main adrenergic, though there are some which are cholinergic; those in the dog are entirely cholinergic; those in the hare are certainly not cholinergic but it is uncertain whether they are adrenergic.

It is interesting to compare the effect of adrenaline after the administration of ergotoxine in the different species with the effect of stimulation. Only in the rabbit and the cat does the effect correspond with the innervation; in the monkey an adrenaline reversal occurs, but the effect of stimulation is paralysed and not reversed; in the dog an adrenaline reversal is obtained which is unaffected by atropine, but the effect of stimulation although reversed is abolished by atropine; in the hare an adrenaline reversal is not seen, but the dilator component of the response to stimulation is abolished earlier than the constrictor component. Evidently the action of adrenaline after ergotoxine has little relation to the action of sympathetic vaso-dilator fibres.

The variation in the action of vaso-dilator substances in the different species is in some ways surprising. The hare resembles the rabbit in that

histamine has no depressor action and its vascular effect is constrictor; acetylcholine, adenosine and small doses of adrenaline exert a powerful dilator action in the muscles of the hare, as they do in the muscles of the dog, but they have little effect in the rabbit. The monkey is peculiar in that acetylcholine has a very slight depressor effect, and in this animal the only efficient dilator substance is histamine.

On the whole the results give some support to the hypothesis that sympathetic vaso-dilator fibres acquire importance in animals which are capable of prolonged exertion. Certainly their absence in the monkey makes it clear that they are not a mechanism which develops according to the position of the animal in the evolutionary scale. Of the five species examined, sympathetic vaso-dilator effects are most easily seen in the dog and the hare, and these are the two which can run the longest distances.

Some special reference should be made to the action of ergotoxine in the hare, for it does not conform with the view that ergotoxine always paralyses sympathetic constrictor fibres, leaving the dilators unaffected. It has been known for some time that ergotamine diminishes the inhibitor action of adrenaline on the intestine and uterus [Rothlin, 1929]. On the whole, however, Dale's [1906] explanation that ergotoxine affects only the motor fibres has been confirmed by later work, and our own observations in the dog [1935] were in complete agreement with it. In the hare, the dilatation caused by sympathetic stimulation disappears when ergotoxine is given, before the constriction is greatly reduced; moreover, ergotoxine causes a progressive fall in the blood pressure, and does not exert the pressor effect seen in the spinal cat. From this it might be concluded that ergotoxine had some paralytic action on the structural elements of the vessel walls of the hare which it does not exert on those of the cat and the dog. Dilatation is, however, readily produced in the hare by adenosine after ergotoxine is given, and the progressive fall of the blood pressure may be due to a weakening of the heart. We must conclude that ergotoxine has a selective action in abolishing the dilatation which forms part of the response to stimulation of the sympathetic chain; it does not prevent all forms of dilatation. It is curious that cocaine abolishes the dilatation due to stimulation; cocaine is further peculiar in the hare in that it does not abolish the pressor effect of tyramine. Evidently the action of ergotoxine and cocaine on the response produced by other drugs varies very much in different species. There is the possibility that the humoral transmitter of vaso-dilator impulses in the hare, which is unlikely to be acetylcholine since the effect of these impulses is not modified by eserine or by atropine, is not adrenaline either. Small doses of adrena-

line have a vaso-dilator action which is followed by a vaso-constrictor action when these doses are increased. When ergotoxine is given the vaso-constrictor effect is paralysed, but the initial vaso-dilatation remains; the action on the response to stimulation is the opposite of this which makes it difficult to believe that the transmitter is adrenaline.

Summary

1. When the sympathetic chain of the wild hare is stimulated the effect on the vessels of the muscles of the hindlimb is not a simple constriction as in the wild rabbit.

Short stimuli cause as their main effect a vaso-dilatation which occurs the moment the stimulus stops and which seems to be superimposed upon a vaso-constriction.

2. The vaso-dilatation is not increased by eserine and is unaffected by atropine; it is, however, abolished by small doses of ergotoxine which do not appreciably reduce the vaso-constriction. Larger doses of ergotoxine reduce the vaso-constriction also. Cocaine abolishes the vaso-dilatation, possibly by intensifying the period of vaso-constriction.

3. The vessels of the muscles of the hare respond far more readily to the action of vaso-dilator substances than do those of the rabbit. Small doses of adrenaline, of acetylcholine and also doses of adenosine produce vaso-dilatation which is much greater than that seen in rabbits of similar size. Acetylcholine is the only substance which has appreciable dilator action in the rabbit. Histamine is vaso-constrictor in the hare as in the rabbit.

4. There are no vaso-dilator fibres in the sympathetic chain of the monkey. Administration of ergotoxine merely paralyses the effect of stimulation and does not reverse it, though it reverses the constrictor action of adrenaline.

5. Histamine is an efficient vaso-dilator substance in the muscles of the monkey, but adenosine has little effect and acetylcholine almost none. Large doses of acetylcholine have scarcely any depressor action in the anæsthetized monkey.

6. The effect of stimulating the sympathetic chain of the greyhound is similar to that in other dogs; there is not an unusually high proportion of vaso-dilator fibres in the greyhound compared with other breeds of dog.

7. The importance of sympathetic vaso-dilator fibres is discussed.

The expenses of this work have been borne in part by a grant from the Government Grant Committee of the Royal Society.

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THE INDUCTION OF PSEUDO-PREGNANCY IN THE RAT BY ELECTRICAL STIMULATION THROUGH THE HEAD

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(*Received* 11 *August* 1936)

The sex cycle of the rat was first accurately described by Long & Evans [1922]. By the use of the vaginal smear technique, they showed that the average length of the œstrous cycle is 4–5 days. This cycle could be prolonged in two ways, first by pregnancy and secondly by the pseudo-pregnancy which followed sterile copulation and mechanical stimulation of the cervical canal of the uterus. The average duration of pseudo-pregnancy was found to be 14 days.

Shelesnyak [1931] showed that pseudo-pregnancy in the rat could also be obtained by electrical stimulation of the cervix of the uterus.

It is probable that the mechanism whereby these stimuli produce pseudo-pregnancy in the rat is similar to that producing ovulation and pseudo-pregnancy in rabbits, ferrets, and perhaps cats. It has been shown that the nervous connexions of the ovary are not of direct importance, for the reactions are obtainable after transplantation of this organ [Long & Evans, 1922, for rats; Asdell, 1926, and Friedman, 1929*a*, for rabbits]. Zondek & Aschheim [1927] showed that implantation of anterior pituitary tissue into mice produced ovulation. Bellerby [1929] and Friedman [1929*b*] obtained the same result by injection of anterior pituitary extract or urine of pregnancy into rabbits, whilst McPhail [1933] showed that similar injections of pituitary extract induced ovulation in the ferret. Conversely Fee & Parkes [1929] demonstrated that rabbits, hypophysectomized within 1 hour of copulation, did not ovulate 9 hours later. Thus there is little doubt that these ovarian changes occur through the intermediation of the anterior lobe of the pituitary gland.

The manner in which the various stimuli produce their effect on the pituitary is not so well known. Artificial insemination, injection of

extracts of vaginal plugs, seminal vesicles and prostate glands, and injection of extracts of the cervical canals fail to have any effect on the ovaries [Hammond, 1925; Vogt, 1933; Freyer, 1921], showing that the reaction is not caused through absorption of substances into the blood stream, either from the seminal fluid or female tract. It appears more likely that the pituitary is stimulated nervously. The evidence for this is that electrical stimulation of the cervical canals can produce pseudo-pregnancy in rats [Shelesnyak, 1931], and that general anæsthesia decreases the percentage of positive results, both to mechanical stimulation [Leonard *et al.* 1929], and also to electrical stimulation of the cervix [Haterius, 1933]. It is possible that the sympathetic system plays a part in stimulating the pituitary gland [Haterius, 1933; Friedgood & Pincus, 1935], though it is far from being the whole of the nervous element involved [Hinsey & Markee, 1933; Vogt, 1931, 1933; Brooks, 1935; Cannon *et al.* 1929].

Lately, Marshall & Verney [1936] have added further evidence that the effect on the pituitary gland is nervous. They obtained ovulation and pseudo-pregnancy in the rabbit by electrical stimulation of the lumbar spinal cord and head. The stimulation was strong and of a diffuse nature, but it is to be presumed that they were stimulating some part of the nervous pathway to the pituitary gland. On the grounds of the previous assumption that pseudo-pregnancy in the rat is produced by a mechanism similar to that giving rise to ovulation and pseudo-pregnancy in the rabbit, it was thought that stimulation through the head and spinal cord of the rat might initiate the pseudo-pregnant condition. This has proved to be correct, at least for stimulation through the head.

Methods

Sexually mature female rats were isolated and had their œstrous cycles plotted, by daily observations of the vaginal smears. When the cycles were of a normal rhythm, the animals were stimulated, under ether anæsthesia, at the stage of œstrus assessed by the presence of cornified cells only in the smear. The stimulus used was an alternating current of 50 cycles, with a voltage varying between 20–40 volts. The time of stimulation was 3 sec. on, 7 sec. off, 3 sec. on, and then this was repeated after an interval which varied between 5 min. and 24 hours.

In the head stimulation, the electrodes were a saline swab in the mouth, and a needle electrode under the skin at the nape of the neck. In the spinal cord stimulation, one electrode was placed in the rectum, and the other under the skin over the lumbar vertebræ.

The shock of the stimulation was very great, giving a generalized convulsion, affecting every part of the body. Respiration generally ceased, but began again after artificial application. In three cases, head stimulation killed the rats, and in two more produced paralysis of the posterior half of the body. Apart from these five cases though, complete recovery occurred within 15 min.

Results of head stimulation

Twenty-one rats have been stimulated through the head, many of these being used more than once, after allowing time between the stimuli for the cycles to regain their normal rhythm. Forty-three stimuli applied at various stages of œstrus gave twenty-eight positive responses (delays in the cycle of 10 days or over), thirteen negative results (no interference with the regular œstrous cycles), and two inconclusive results (delays between 7 and 9 days). These delays are counted from the metœstrus following stimulation to the next period of œstrus. The number of positive reactions is thus 68 p.c.

Some difficulty was at first experienced in deciding whether the delayed cycles were true pseudo-pregnant responses and not merely states of anœstrus initiated by the great shock. This latter was felt to be unlikely, since in the great majority of cases such perfect recovery was obtained, but it was, nevertheless, a possibility.

The ovarian cycle in the rat is very difficult to follow, either macroscopically, or by use of the common histological techniques. The corpora lutea formed by the rhythmical ovulation at each stage of œstrus, though functional for only a very short time, are anatomically persistent, with little diminution in size, for many days. Thus twenty to thirty corpora lutea are usually to be found in a normal rat's ovary.

The uterine changes were first investigated. Allen [1931] described the cyclic changes in the rat's uterus occurring throughout the œstrous cycle, pseudo-pregnancy and pregnancy. He stated that commencing on the ninth day of pseudo-pregnancy, the endometrium, which previously possessed a smooth surface, develops crypts and becomes hillocky. He also pointed out that this reaction is not sufficiently marked to use as a test for the potency of corpus luteum extracts. From very limited data the crypts in the endometrium of rats killed in œstrus appeared almost identical with those of rats killed from the ninth to twelfth days of the delayed cycles. One possible cause of this discrepancy lies in the method of fixation. Allen employed Bouin's solution as a fixative, whilst in this

work 10 p.c. formalin was used. As this reaction appeared so indefinite, other methods were sought.

That the delayed cycle was a true pseudo-pregnant response was finally shown by the following facts:

(i) The delay, if it occurred at all, lasted, in every case except two, between 9 and 16 days, which is the average duration of pseudo-pregnancy.

(ii) The production of deciduomata. Two silk threads were inserted into one uterine horn on the fourth or fifth days following stimulation, the animals being killed on the ninth or tenth days. In fourteen cases out of fifteen, marked deciduomata were produced. These were fixed in 10 p.c. formalin and stained with hæmatoxylin and eosin, or else fixed in Heidenhain's "Susa" mixture and stained by the method described by Selye & McKeown [1935]. The structure of the deciduomata produced in these experiments is identical with the structure of those produced during pseudo-pregnancy following sterile coitus, or glass-rod stimulation of the cervix.

(iii) The ovaries were taken from nine rats, generally on the tenth day after stimulation, and stained for lutein-lipoid droplets. The method used was similar to that described by Long & Evans [1922, p. 39]. In their discussion of the lipoid droplets in the cells of the corpora lutea, Long & Evans adduce evidence that active corpora possess lipoid droplets which are small and uniform in size, and that the total quantity of lipoid is moderate in amount. At the end of the functional life of the corpora the granules increase in size and become irregular, whilst the total quantity of lipoid also markedly increases before final regression sets in. In all the ovaries taken towards the end of the delayed cycle could be distinguished old, non-functioning corpora lutea in various stages of degeneration, and active corpora containing lipoid droplets of uniform size.

(iv) In two cases sections were made through mammary tissue, taken 10 and 11 days after stimulation. In both animals small quantities of milk were found to be present in the alveoli.

In view of the above facts, it was felt safe to conclude that the stimulation had produced a true pseudo-pregnancy in 68 p.c. of the animals. This rough figure was obtained using stimuli of varying strengths and time relations, and it is possible that after further work on the optimum conditions, a slightly higher percentage could be obtained. The most constant results were obtained with a potential difference of 30 volts and a time interval between the two sets of stimuli of 1–1½ hours.

It is convenient to mention at this point, that, in the case of one rat, stimulated through the head eleven times at different stages of œstrus, only one pseudo-pregnancy was produced. All the vaginal smears of this animal were dry and scanty, and even the dioestrous smears contained few leucocytes. This rat was eventually killed when in œstrus. On post-mortem, the ovaries were observed to be bound to the uterine horns by much connective tissue, and on sectioning were found to contain many ripe follicles, but only a few degenerate corpora lutea. Most of the previous cycles must have been lacking in ovulation and consequent formation of luteal tissue. Since the only positive response was obtained from the first of the eleven stimuli, it may be that during this attempt some part of the nervous mechanism underlying ovulation and pseudo-pregnancy was damaged. On the other hand, it has been noted by several workers that such cycles do appear in rats and mice that are otherwise apparently normal [Parkes, 1929]. The results obtained from this animal have not been included in any of the above figures.

Results of spinal cord stimulation

Seventeen stimuli were applied to thirteen rats at different stages of œstrus, giving thirteen negative and four inconclusive results. These latter results, and also the two similar ones obtained by head stimulation, are abnormally short pseudo-pregnancies.

The normal duration of pseudo-pregnancy in the rat has been set by different observers at 8–23 days, average 14·38 [Long & Evans, 1922]; 9–21 days, average 14·03 [Shelesnyak, 1931]; 7–19 days, average 14·53 [Slonaker, 1929]. These six delays then, one of 7 days, four of 8 days and one of 9 days, are within the minimum limits as set by these authors, and thus may be normal in character. It is unlikely, however, that the only four positive results obtained by spinal cord stimulation should all be short, if normal, when the great majority of pseudo-pregnancies persist for approximately 14 days.

It is worthy of note that Marshall & Verney found the pseudo-pregnancy in one of their rabbits, kept alive for 14 days after a spinal cord stimulation, to have come to an end a few days sooner than usual.

Rugh [1934] observed that, in frogs, ovulation does not obey the all-or-nothing principle, but is a graded reaction. It is likely that in some Mammalia ovulation and pseudo-pregnancy are also graded reactions, and that, whereas the natural stimuli for these effects, such as sterile copulation, are maximal stimuli, some of the cruder artificial forms of stimulation are submaximal.

Discussion

Concerning the head stimulation, the rough figure of 68 p.c. positive results seems in accordance with the results quoted by previous workers on the induction of pseudo-pregnancy in the rat by artificial means. Thus after glass-rod stimulation of the cervical canal, Long & Evans [1922, p. 80] found 68 p.c. positive results, Leonard *et al.* [1929] obtained 72 p.c., whilst Slonaker [1929] gave lower figures. By electrical stimulation of the cervix uteri, Shelesnyak [1931] obtained 82 p.c. positive responses, and Haterius [1933] 92 p.c., whilst the latter by the mechanical stimulation found 47 p.c.

It is of interest to note that sterile copulation gives uniformly positive results according to Haterius [1933]. He suggests that there is a "psychic factor" present in the copulation, which is of course missing in the forms of artificial stimulation so far used. If this view is adopted, one might assume that the higher centres also influence the anterior lobe of the pituitary gland by some nervous pathway and, further, that these nerve fibres would be stimulated by the large shocks applied through the head. In this case, one might expect the same results as are obtained by sterile coitus. That this is not so can hardly be taken as evidence against the presence of the "psychic factor", owing to the complete lack of knowledge as to the excitability of this hypothetical pathway.

Further attempts have been made on rabbits to localize the site of action of this type of stimulation, and at the present time it appears likely that the results may have been due, in part anyhow, to an effect on nerve fibres in the hypothalamus, which are passing to the pituitary gland via the infundibulum. These latter results will be published shortly.

Although very unlikely, it is just possible that ovulation following central nervous stimulation in the rabbit is due to a direct nervous action on the ovaries. It is well known that sections of the spinal cord in monkeys will affect the ovarian cycle [Zuckerman, 1934; Markee *et al.* 1936]. Thus the state of an ovary can be altered by nervous influences. Once ovulation had occurred, then, according to the work of O'Donoghue [1913] who showed that formation of corpora lutea followed the artificial rupture of large follicles in the ovaries of rabbits, pseudo-pregnancy would follow. Although this is a very improbable explanation of the observed effects, it is possible to account for the results of stimulation in the rabbit, without taking the pituitary gland into consideration. But in the case of the rat we are dealing with an animal which ovulates and forms corpora lutea rhythmically, and so it is difficult to see how prolongation of

the life of the corpora lutea could be brought about by a nervous effect on the ovary. The result is almost certainly due to intermediation of the pituitary gland.

Summary

(1) Electrical stimulation through the head of the rat, during œstrus, produces the condition of pseudo-pregnancy in 68 p.c. of cases.

(2) A similar stimulus applied to the lumbar spinal cord gives no definite results.

(3) The response to the head stimulation was shown in several ways to be a true pseudo-pregnant response, the most convincing method being the production of deciduomata.

I should like to express my sincerest thanks to Dr F. H. A. Marshall, firstly for suggesting this problem to me, and secondly for his never-failing advice and encouragement. My thanks are also due to Mr J. Cash for his valuable help in the laborious task of taking daily vaginal smears.

The expenses of this research were in part defrayed by a grant from the Medical Research Council, and by the Marmaduke Shield Scholarship in Anatomy.

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612.621.5:612.463

WATER METABOLISM IN RELATION TO THE MENSTRUAL CYCLE

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(*Received* 28 *July* 1936)

The menstrual cycle in Old World monkeys is often characterized by the regular occurrence of subcutaneous œdema. As a rule the œdematous swelling, or sexual skin, is confined to the circum-anal and circum-genital zones, the form and colour it takes varying from species to species; other regions of the body may also be affected. In certain species the time relations of the swelling are not uncommonly irregular (more particularly during adolescence). In general, however, the maximum swelling occurs about the middle of the cycle, and in regularly menstruating animals belonging to genera such as *Papio*, the baboons, in which the sexual skin is best developed, the time relations of the swelling are definite and fixed. Swelling begins during menstruation, slowly reaches a maximum (at which time the sexual skin appears as a considerable tense "outgrowth"), and then, shortly after the mid-point of the cycle, it dramatically declines, and is quickly resorbed. The resorption is very rapid at first, and in most species all except a somewhat constant residuum of swelling disappears within the course of 2–6 days.

Sexual-skin swelling may be regarded as an œstrous phenomenon comparable with the vulval swelling shown by ferrets in œstrus. The œstrous nature of the swelling is revealed both by the fact that it is usually confined to the pre-ovulation phase of the cycle, and by the observation that the onset of its subsidence in normal animals is correlated with the occurrence of ovulation [Zuckerman, 1930; Zuckerman & Parkes, 1932]. The discovery that the sexual skin of monkeys can be activated by œstrogenic substances [Allen, 1927; Zuckerman & Fulton, 1934, for further references] constituted the first step in our understanding of the sexual-skin mechanism; it still remains the outstanding fact in our knowledge of sexual-skin physiology.

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Such histological observations as have been made suggest that the œdema of the sexual skin represents an intercellular accumulation of fluid. This conclusion is supported by the fact that when a swollen sexual skin is removed at autopsy, incised, and left in a dish, a fairly large amount of clear and moderately thick fluid drips from the exposed surface. In the circumstances it is noteworthy that the swollen skin does not pit on pressure in the same way as does an œdematous ankle in cardiac failure.

The present paper is a report of an initial investigation into the fluid exchange of the sexual skin. The object of the investigation was to discover whether the fluid deposited in the skin is selectively absorbed from an otherwise unaltered volume of body fluid, or whether an extra amount of water is retained during the phase of swelling and eliminated during the period of resorption. Owing to the difficulty of withdrawing blood daily from a monkey, it was decided to approach the problem by studying the animal's daily intake and output of water.

The experimental animal

The animal used in this investigation was a fully mature female pig-tailed macaque (*Macaca nemestrina*) with a well-developed sexual skin. The swelling in this species extends around both the anal and external genital orifices; it has been fully described elsewhere [Zuckerman, 1935]. The time relations of the sexual-skin cycle in this species are similar to those of the Hamadryas baboon (summarized above) except that the phase of swelling is usually a day or two shorter than the phase of quiescence. Apart from its second cycle, in which the phase of swelling was unduly prolonged (presumably because of a cystic ovarian follicle), the eleven cycles during which the experimental animal was under observation were remarkably regular.

Table I. Menstrual cycles of pig-tailed macaque

Cycle	Total length days	Phase of swelling (follicular phase) days	Phase of quiescence (luteal phase) days
1	28	11	17
2	51	35	16
3	29	15	14
4	28	13	15
5	29	14	15
6	31	14	17
7	28	14	14
8	30	15	15
9	34	18	16
10	32	14	18
11	30	14	16

If the second cycle is excluded from the calculations, the mean cycle length is 30 days, the follicular and luteal phases being 14·2 and 15·8 days respectively.

The experimental observations on which this report is based were made during the eighth, ninth and tenth cycles. These cycles averaged 32 days in length, and the mean follicular approximately equalled the mean luteal phase.

Technique

General

The animal was confined in a wire-mesh cage, 3 ft. deep, 2 ft. 3 in. wide, and 3 ft. 6 in. high, from back to front of which passed an iron-grid perch. Like the walls, the floor of the cage was of 1-in. wire mesh, beneath which was a removable draining tray gently sloping on all sides to a central opening fitted with a spout. A jar was placed beneath the spout for the collection of urine. A second, but removable, frame of ½-in. wire mesh was placed between the floor of the cage and the floor of the draining tray, its purpose being to prevent, as far as was possible, pieces of food falling into the tray. The cage was firmly clamped to the ledge on which it stood, so as to prevent the animal rattling the whole structure, and thereby shaking water from the apparatus through which it was provided.

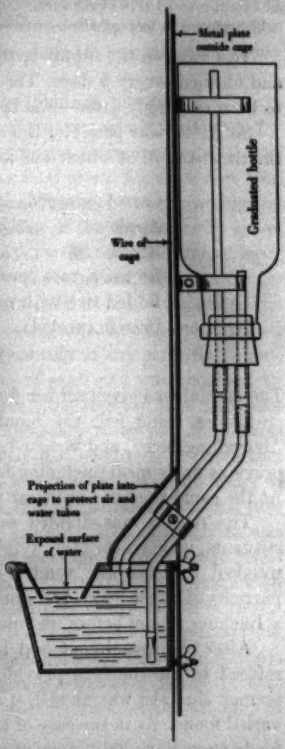
Metal plate outside cage
Graduated bottle
Wire of cage
Projection of plate into cage to protect air and water tubes
Exposed surface of water

Fig. 1. Plan of apparatus for controlling supply of water.

A plan of this apparatus is shown in Fig. 1. It comprised an inverted wide-necked graduated bottle, through the stopper of which passed one glass tube for the passage of air and another for the passage of water. The water tube just penetrated the stopper above; below it reached to the bottom of a drinking trough. The air tube extended from the base of the inverted supply bottle above, to the surface of the water in the trough below. As the animal drank and the water-level fell, air passed into the supply bottle, water flowing

through the water tube until the mouth of the air tube was once again in contact with the water in the trough. The supply bottle was clipped to the outside of the cage, and completely shielded from the monkey by a wide metal plate. The air and water tubes passed through the plate into the trough, which was screwed to the plate from inside the cage. The trough itself was provided with a firm hinged lid which had a central funnel opening 1 in. in diameter. The lid protected those parts of the tubes which had to enter the cage, and since it covered all except a small area of the surface of the water, it also helped to prevent evaporation (which proved to be negligible).

The water in the supply bottle was renewed, and the trough removed and cleaned, every 5 days. The amount of water drunk—which proved to be surprisingly little—was measured daily.

So far as was possible, the animal was given a standard daily diet, the constitution of which was as follows:

	g.
Carrot (scraped)	100
Apple	110
Bread	50
Boiled potato (peeled)	165
Boiled rice with raisins	175
Orange (peeled)	60
Total	660

The animal was given all her food in one meal at 10 a.m.

Before being fed, the monkey was removed from her cage in a "travelling box", and weighed. While in the box, all the residue of her previous day's meal (including fæces) was collected and the cage cleaned. At the same time the urine jar was changed.

The residue was divided as far as possible into its separate constituents, which were then weighed, the unrecognizable fragments being weighed together as "sundry refuse". In order to remove any fine particles of refuse which had drained into it, the urine was filtered through a Büchner funnel before its volume was taken.

After the experiment had been in progress some time, the animal refused to eat the food provided, and for some days, during which it seemed that she was unwell, it was necessary to provide her with more varied foods. As in the case of the constant articles of diet, these special foods were given only in weighed quantities.

The period of observations extended from 18 March to 22 June inclusive. Until the last two days of the experiment, the room in which the monkey was housed was kept, by means of thermostatically controlled electrical tubular heaters, at a temperature which varied between the narrow limits of 65–70° F.

Estimation of water intake

The water contained in her solid food provided most of the fluid absorbed by the animal during the course of the experiment. Frequently she drank nothing for several consecutive days, but on two very hot days at the close of the experiment the temperature of the animal room rose to the region of 80° F., and on each of these days the animal drank about 100 c.c. of water.

The water taken in daily by the animal through her solid foods was calculated, as closely as the conditions of the experiment allowed, by subtracting the water content of the daily residue, corrected for evaporation, from the total water content of the food given for that day. The total water content of the standard diet was obtained by subtracting the weight of a dish containing the dried residue of the food after 24 hours in an air-oven, kept at 120° C., from the initial weight of the dish and food, the observation being repeated four times; the results obtained varied by less than 1 p.c. The water content of each item of food ever given the animal was also estimated on two separate occasions. The figures obtained provided a basis for calculating the water content of any given day's diet.

The water content of the food residue of each day was obtained by the same method of drying. Since, however, the rejected food had been exposed to the air for 24 hours, it was necessary to correct the figures obtained to allow for the evaporation that had occurred. A correction factor was secured by first observing the loss in weight over 24 hours of a known quantity of each separate food, divided as far as possible into pieces comparable with those left by the animal. Each observation was repeated twice. The amounts lost in evaporation by the recognizable items of food left by the animal on 10 days, chosen at random, were then calculated, the ratio of the total loss of each day to the weight of recognizable residue of that day providing an index whereby the evaporation loss of the day's "sundry refuse" could be calculated. The mean of these separate ratios for the 10 days provided a correction factor of 0·2, i.e. 20 p.c. of the total residue of each day represented the amount of water lost through evaporation.

In so far as the crude daily water balance was calculated as the difference between the total amount of water presented to the animal (both as free water and as water contained in her solid food) minus the sum of the urine collected and the water contained in the food residue, any urine caught up in the food residue would be accounted for in the calculation of the daily water balance.

Estimation of water excreted

Repeated observation showed that only about 70 p.c. of a quantity of 210 c.c. of urine poured into an empty cage, similar to the one used in the experiment, drained into the collecting jar. The remaining 30 p.c. held to the wire mesh and to the floor of the draining tray, and presumably evaporated. It was necessary, therefore, to correct the daily readings by adding 43 p.c. to the actual readings obtained. Although it cannot be held that this figure represents the actual amount lost in this way every day, it is plain that its employment as a correction factor could not in any way obscure cyclical variations either in the urinary excretion or in the daily water balance.

Urine lost through absorption by food residues in the cage has already been considered. Water lost daily by way of the fæces was accounted for daily as the fæces were included with the food residue.

Estimation of water balance

The daily water balance was calculated as the difference between the sum of (*a*) the water drunk by the animal, (*b*) the water presented to the animal in her solid food, and (*c*) the water of oxidation, on the one hand, and the sum of (i) the water contained in the food residue (corrected for evaporation), (ii) the urine (corrected for loss), and (iii) the water lost insensibly by way of skin and lungs, on the other.

Water of oxidation. This was calculated, with the help of the tables provided by Plimmer [1921], on the basis of the amount of food consumed on the 10 days chosen for the estimation of the water lost by evaporation of the food residues. The mean daily water of oxidation for the 10 days (during which the animal was on her standard diet) proved to be 67·6 c.c.

Water lost through lungs and skin. The amounts of water lost in this way daily during the 10 days referred to above were obtained by the use of the formula: Cal.* = 2·42 I.W. [Peters, 1935], where I.W. is "insensible water". This formula, according to Peters, applies fairly generally to mammals.

The daily amounts of protein, carbohydrate and fat were calculated from tables, and their calorific value determined by the customary method. The figures obtained varied between 365·8 and 617·7 Cal., the mean being 531·9 Cal. Further calculation showed that the approximate mean calorific value of the food consumed by the animal was 1 Cal. per g.,

* Cal. = 1000 small calories.

and corrections were then made to allow for the "sundry refuse". The corrected figures, which are given in Table II, varied between 325·9 and 596·5 Cal., the mean being 494·5 Cal. It is noteworthy that this figure is well above the basal heat production of monkeys as given by both Bruhn [1934] (653 Cal. per sq. m. in 24 hours), and Rakitien [1935] (608 Cal. per sq. m. in 24 hours). On the basis of their results the basal

TABLE II. Calorific intake of pig-tailed macaque on 10 days chosen at random

Day	Cal.
1	325·9
2	508·4
3	487·4
4	487·3
5	557·8
6	474·1
7	466·1
8	596·5
9	546·0
10	495·0
Mean	494·5

metabolism for the pig-tailed macaque which was the subject of the present experiment should be in the neighbourhood of 200 Cal. (Body surface calculated from the formula $S = KW^{\frac{2}{3}}$ [Meeh, 1879], K being 11·7 for the macaque [Lee and Fox, 1933].)

From the relation Cal. = 2·42 I.W., it follows that the insensible daily water loss is about 204 g.

EXPERIMENTAL RESULTS

(*a*) *Daily fluctuations in weight*

The animal was weighed daily, beginning on the day following the onset of sexual-skin subsidence in the first cycle of the experiment. The records obtained are plotted in Fig. 2, and show that the animal's weight rises in the first half of the cycle, when the sexual skin undergoes its phase of swelling, and falls during the second half, when the sexual-skin swelling is resorbed.

The maximum weight of the animal during the first cycle, taken on the day following the first sign of sexual-skin subsidence, was 6060 g., and the minimum weight reached during the ensuing phase of quiescence was 5150 g. During the second cycle, when the animal was unwell, the minimum weight when the skin was inactive was 4810 g. and the maximum weight at the height of swelling 5150 g. In the third cycle the corresponding weights were 4860 and 5660 g. respectively. While it cannot be assumed, in the absence of knowledge about cyclical variations in the

weights of other tissues, that the cyclical increase in the weight of the experimental animal is entirely due to swelling of the sexual skin, it is justifiable to regard the increase as being mainly a result of the swelling. Assuming the animal to be in normal health, therefore, and to be fully grown, the differences between these figures for each cycle should give an approximate measure of the sexual-skin œdema. The differences for the first, second and third cycles are 910, 340 and 800 g. respectively. The

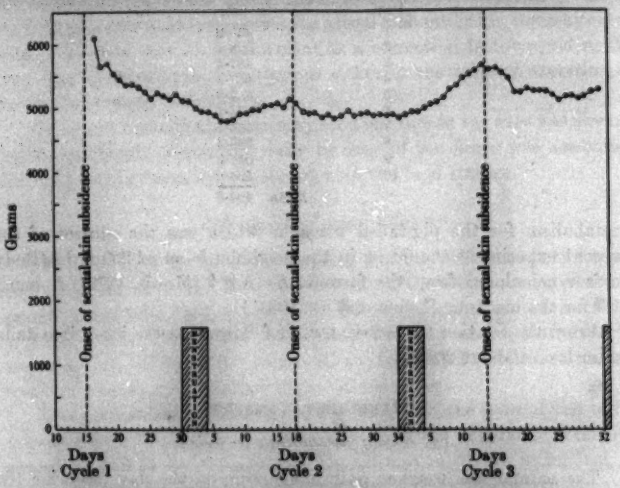

Fig. 2. Daily variations in the body weight of the pig-tailed macaque.

small value for the second cycle is explained by the fact that during this cycle, when the animal "went off her food" and was clearly ill, the swelling of her sexual skin did not reach normal proportions.

The average minimum weight of the animal during the three experimental cycles was 4940 g. The animal had also been weighed daily for a period of 4 months, ending 12 weeks before weight records were kept in this experiment. The average minimum weight per cycle during this earlier period was 4655 g., and the maximum weight reached during a phase of sexual-skin swelling was 5900 g. These several observations show that the monkey was not increasing in weight significantly during the course of the 9 months preceding the close of the experiment. The

mean minimum weight for the animal during the follicular and luteal phases of the second cycle (when the animal was ill) was 4825 g. A corresponding figure for the first and third cycles is 5070 g. About 5000 g. can therefore be justifiably regarded as the animal's normal weight during phases of sexual-skin quiescence. The mean increase in weight during the first and third cycles, when swelling of the sexual skin was normal, was 855 g. It follows, therefore, that the œdema which occurs during the follicular phase of the menstrual cycle can amount to more than 17 p.c. of the animal's normal weight. As argued above, it may safely be assumed that most of the œdema is concentrated in the sexual skin. This figure may be compared with the following, recorded during post-mortems of baboons. In two mature *Papio hamadryas*, autopsied when swelling was at its maximum, the sexual skin alone weighed 10 p.c. of the total body weight (10 kg.). In a specimen of *Papio porcarius* which showed an abnormal degree of swelling, associated with irregular cycles, the sexual skin made up 28 p.c. of the total body weight.

In the second and third cycles, where the data allow of comparisons, the onset of the decline in body weight agrees to within a day with the onset of sexual-skin subsidence, as determined by direct observation. In the first cycle, body weight fell rapidly immediately after the onset of sexual-skin subsidence, and then slowly for 13 days. In the second cycle, the initial fall was again rapid, but only 7 days passed before a minimum weight was reached. And in the third cycle the fall was again rapid at first, after an initial fluctuation of weight, and 12 days passed before a minimum weight was reached. These observations agree closely with direct observations of the sexual-skin changes. Daily fluctuations in body weight, of a maximum of 80 g., and in trend against the general trend of the weight curve, were also observed.

Body weight rose 100 g. during the last two days of the third cycle. The magnitude of this rise, the fact that it was continued on the first day of the ensuing cycle, and the fact that its occurrence was against the trend of the general weight cycle, suggest that the increase should be regarded as a general increase in somatic weight. It was noted before that the room temperature was much higher on these two days than during the rest of the experiment, and that the monkey also drank much more on these two days. Analysis of the water balance shows that the animal was retaining an average of 50 g. more water on these two days than she had been doing in the preceding sixteen.

(b) Variations in the water intake

Fig. 3 gives a record of the daily fluctuations in water intake that occurred during the period of the experiment. The daily variations are large, and the available data do not allow of their analysis. While there

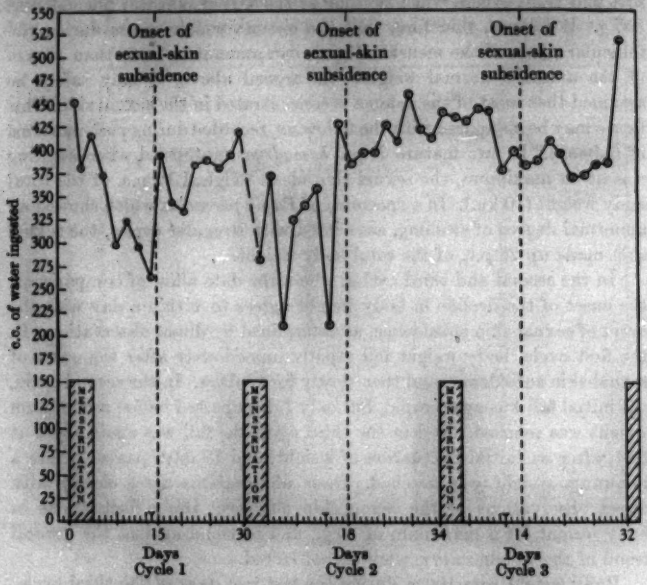

Fig. 3. Daily variations in the water intake of the pig-tailed macaque.

is no clear cyclical fluctuation in intake which can be correlated with the sexual-skin cycle, there does seem to be some indication that the amount of water consumed falls during the phase of swelling and rises during the

TABLE III. Mean daily water intake during phases of menstrual cycle

	Phase of swelling c.c.	Phase of quiescence c.c.
First cycle	343	376
Second cycle	314	411
Third cycle	421	403
Average	359	397

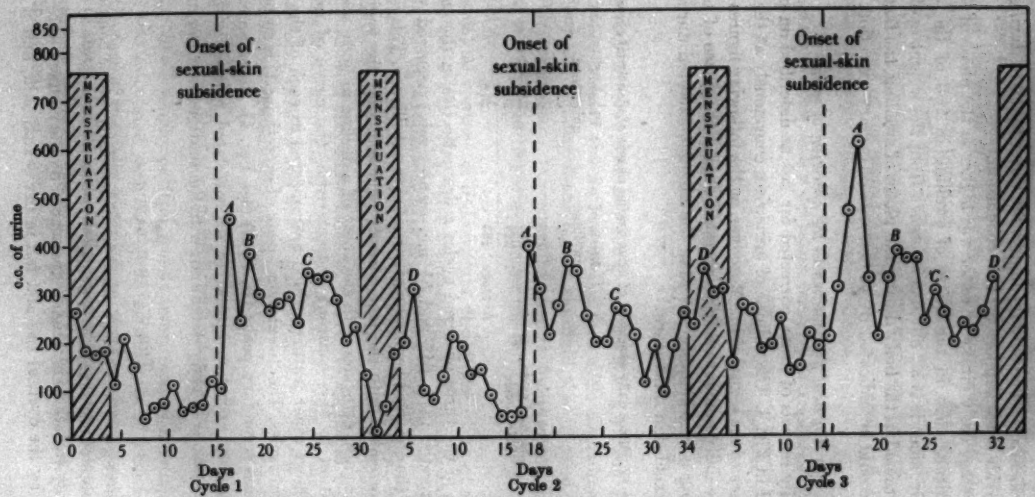

Fig. 4. Daily variations in the amount of urine excreted by the pig-tailed macaque.

phase of subsidence. The average figures for water taken in during these phases provide some support for this conclusion.

The average daily intake of water was higher in the phase of sexual-skin subsidence than in the phase of swelling in two of the cycles. Accordingly, since it is an obvious inference from the weight cycle that water is retained in the body during the phase of swelling, the fact of its retention should be indicated by the records of urinary excretion.

(c) *Cyclical fluctuations in the excretion of urine*

The daily records of urine, corrected for loss as described on p. 374, are charted in Fig. 4. The volume of urine rises enormously at the onset of subsidence of the sexual skin. Moreover, as the following figures show, the average daily amount of urine excreted during the phase of sexual-skin swelling is considerably less than the amount excreted during the phase of sexual-skin quiescence.

TABLE IV. Mean daily excretion of urine during phases of menstrual cycle

	Phase of swelling c.c.	Phase of quiescence c.c.
First cycle	125	286
Second cycle	145	232
Third cycle	224	307
Average	165	275

In each of the three observed cycles a dramatic rise in the amount of urine excreted (342, 645 and 51 p.c. respectively) occurred within 24 hours of the observed beginning of sexual-skin subsidence. This finding is clearly correlated with the fact, already recorded, that the beginning of subsidence also coincides with the onset of a decline in body weight.

In addition to this constant characteristic, the curve of urinary excretion shows other features which are repeated in each of the three experimental cycles. The "ovulation" peak of excretion (*A*) is succeeded by three main secondary peaks (*B*, *C*, *D*) (see Fig. 4). These secondary peaks of urinary excretion appear to be correlated neither with any specific fluctuations in body weight nor with fluctuations in water intake.

THE WATER BALANCE

The figures given in Table V show the mean daily water balance for the two phases of the sexual-skin cycle.

If the only variations in weight experienced by the monkey were those occasioned by the cyclical rise and fall in her sexual skin, and if there

were no other factors present to effect gross disturbances in water metabolism, it should be expected that the average daily positive water balance of the phase of sexual-skin swelling should equal the negative water balance of the phase of subsidence. That the figures we have obtained for the first and third cycles agree with this postulate to the extent that they do indicates that our methods provide reasonable approximations of the actual magnitudes of the separate factors which enter into the estimation of the water balance.

TABLE V. Mean daily water balance during phases of menstrual cycle

	Phase of swelling c.c.	Phase of quiescence c.c.
First cycle	+74·6	−47·4
Second cycle	+32·6	+42·6
Third cycle	+64·6	−38·4
Average	+57·3	−14·4

Calculation shows that only 408 g. or 3·2 p.c. of a total of 12,813 g. of water consumed by the animal in the first cycle (including water of oxidation) are not accounted for by the fluid she excreted (including insensible water loss). Corresponding figures for the second cycle are 1268 g., or 9·1 p.c. of 13,904 g., and for the third cycle 213 g., or 1·4 p.c. of 15,311 g.

The actual increase in body weight during the phase of swelling in the first cycle was 910 g. Our estimate of the total amount of water retained during this phase is 1119 g. Corresponding figures for the second cycle are 340 and 587 g.; and for the third cycle 800 and 904 g. Considering the unavoidable imperfections in the technique followed in the determination of the water balance, the correspondences between our estimates of the amounts of water retained in the body during the phases of sexual-skin swelling and the actual increases in body weight during these phases are remarkably close.

DAILY VARIATIONS IN FOOD INTAKE

The "dry" weight of food (solids minus water) consumed by the animal was calculated each day from the amounts of fresh food eaten. As in the case of the daily water intake, there is some indication, as the figures (Table VI) of the mean daily consumption show, that the daily dry food intake is greater in the second half of the cycle than in the first half when the sexual skin is active.

TABLE VI. Mean daily consumption of food ("dry" weight) during phases of menstrual cycle

	Phase of swelling	Phase of quiescence
	g.	g.
First cycle	95	105
Second cycle	75	112
Third cycle	123	125
Average	98	114

DISCUSSION

The fluctuations in body weight and in the amount of urine excreted which occur in relation to the cyclical swelling and subsidence of the sexual skin imply that the menstrual cycle in the pig-tailed macaque is directly correlated with cyclical fluctuations in the daily water balance. This implication is borne out by our figures for the first and third cycles; the discordance of our data for the second cycle may be attributed partly to the illness of the experimental animal during this period and partly to imperfections in the estimation of the daily water balance in monkeys, which the nature of these animals renders unavoidable. Because of these imperfections, it is a noteworthy fact that there is a relatively close correspondence between the actual observed rise in weight due to the deposition of fluid, presumably in the sexual skin, and the increase in weight calculated on the basis of the daily water balance. The mid-interval onset of sexual-skin subsidence, which, on the basis of studies of the baboon, is probably coincident with ovulation (when it occurs), agrees to a day with a sharp increase in the amount of urine excreted as well as with a fall in body weight, and both the latter two phenomena thus appear to be indirectly correlated with the ovarian cycle.

An apparent anomaly is presented by the fact that slight, but nevertheless recognizable, cycles in water intake and food consumption run counter to the more pronounced cycle of urinary excretion. When, as is shown by the fluctuations in the daily water balance and body weight, water is being held back in the tissues, the animal as a rule consumes less fluid and food than when the sexual skin is returning its stored water to the general circulation to be excreted. Since the major part of the water consumed by the animal is provided by its solid food, there is a direct relation between the contrary cycles of water intake and dry-food intake. Moreover, although the water intake during the first half of the cycle may be less than in the second, it is plain that the amount ingested in the first half is sufficient to allow for the deposition of fluid in the sexual-skin swelling.

The swelling of the skin is immediately determined by an œstrogenic agent, which appears to have a selective action upon its subcutaneous and cutaneous tissues. Since the denervated sexual skin reacts to œstrogenic substances [Zuckerman, 1935], the site of action of the œstrogen must be either the capillary wall or the extra-capillary tissue, and its initial effect is to promote a considerable flow of fluid into the intercellular spaces of the sexual skin. At the middle of the cycle the amount of œstrogen available is considerably reduced [Zuckerman, 1930, 1936], and as a result the sexual-skin fluid is freed, and rapidly returns to the blood stream, from which it is immediately removed in large quantities by the kidneys. The average daily amount of urine excreted in each of the first three days following the onset of sexual-skin subsidence in the three experimental cycles was 420, 305 and 458 c.c. respectively, amounts which represented a daily loss of 7, 6 and 8 p.c. of the maximum body weights reached during the phases of sexual-skin swelling. It would seem that the animal immediately over-compensates for the pronounced loss of fluid both by a lowering in the level of urinary excretion and, as is shown by the increased water intake after subsidence of the sexual skin begins, by consuming more fluid. An excess of body fluid would nevertheless still prevail, and further phases of increased urinary excretion, alternating with phases in which urine was excreted at a lower level, would occur until a volume of body fluid remained which was representative of the phase of sexual-skin quiescence. Judging by the high level of urinary excretion at the beginning of a menstrual cycle, it would seem that the excess of body fluid of the immediately preceding cycle is not eliminated before that cycle ends. It would also seem that the œstrogenic stimulation of the follicular phase of the cycle is but slight at the beginning of the phase, and that at first the sexual skin is not sufficiently influenced to absorb the excess of body fluid remaining from the previous cycle.

The fact that the fluid stored in the sexual skin during the first half of the cycle is eliminated during the latter phase of the cycle would appear to be related to the times when œstrogenic substances are excreted in the urine. The menstrual cycle of monkeys has not, to our knowledge, been investigated from this point of view. The chimpanzee has been studied by Allen *et al.* [1936]. Œstrogenic substances appear to be excreted in varying amounts at all times of the cycle. The minimum of excretion occurs at the time of menstruation, and the maximum when the sexual skin is either in the last phases of swelling, or beginning to subside ("during or just following the follicular phase of the menstrual cycle").

While the lack of detail in the account from which we are quoting precludes close comparison, it would seem that the high level of excretion during the phase of sexual-skin subsidence in the chimpanzee roughly corresponds to a main phase of excretion of œstrogenic substances in the human cycle. According to Frank [1935], there are two periods of maximum excretion in Man, the first corresponding to the time of ovulation (i.e. the end of the follicular phase), and the second occurring in the week preceding the menses. These two peaks of excretion in women appear to be coincident with peaks of urinary excretion in the course of the resorption of the sexual skin of the monkey. Certain observations are available [Fisher *et al.* 1936] which show that œstrogenic substances may be deposited in the active sexual skin. It is thus reasonable to speculate that the œstrogenic substance excreted by women is liberated at the time of ovulation from tissues in which it has been "deposited" in the pre-ovulation phase.

"The chronological correspondence of increase in the œstrogenic factor in the blood and increase of excretion in the urine" [Frank, 1935] would also appear to be significant from this point of view. The usual interpretation of this correspondence is that œstrogenic substances are secreted at a high rate at the times of their increased concentrations in the blood and urine, and this interpretation is usually put forward as an obstacle to the view that uterine bleeding occurs only at times of low effective œstrogen concentration. An alternative interpretation, which is compatible with this view of uterine bleeding, is, however, indicated by the facts.

Œstrin produces its positive morphological effects during the first half of the cycle. At that time little œstrogenic substance can be detected in the blood [Frank & Goldberger, 1935]. Moreover, it has been found experimentally that œstrogenic substance injected into the blood stream of rabbits cannot be detected in the blood after an interval of 30 min. following the injection [Frank *et al.* 1932]. These facts suggest that œstrin, during the phase it functions physiologically, is withdrawn so rapidly from the blood stream as almost to preclude its recognition there. The fact that œstrogenic substance is more readily found in the blood stream in that phase of the cycle when its positive physiological effects are minimal suggests that the converse of this proposition may also be true, i.e. that the presence of œstrogenic substance in the blood stream during the menstrual cycle is partly indicative of its non-effectivity and of its imminent excretion. In this connexion it may be noted that a corpus luteum is present in the post-ovulation phase of normal cycles,

and that there is some evidence that one of the effects of the corpus luteum is to increase the excretion of œstrogenic substance [Smith & Smith, 1931].

Although the present investigation plainly shows that the fluid deposited in the sexual skin is accounted for by exogenous water, it is nevertheless conceivable that variations would occur in the hæmoglobin index and in the concentration of blood cells if the sexual skin returns water to the blood more quickly than the extra fluid is removed by the kidneys. No studies of this kind have as yet been made on monkeys. Cyclical variations in the constituents of the blood, correlated with phases of the menstrual cycle, have however been reported for women, but the data are very conflicting. Most authors [Pölzl, 1910; Gumprich, 1913; Rowe & Guagenty, 1934] describe a drop in the red cell and hæmoglobin counts during menstruation, and according to Piney [1931] the drop may amount to as much as a quarter of the normal number. Reports that there is a premenstrual rise in the red cell count [Pölzl, 1910; Detre, 1928] are denied by Merletti [1914] who describes a fall at that time. On the other hand both Reich & Green [1932] and Smith [1936] believe, in opposition to most authors, that variations in the red cell or hæmoglobin counts are not correlated with the menstrual cycle. Smith & McDowell [1929] have also found that the rhythms in the white cell count are not affected by menstruation.

The occurrence of cyclically fluctuating œdema in women has also been reported. The dependence of such œdema on the ovarian cycle is made clearer by observations of Veil & Bohn [1922], to the effect that the body weight rises during the administration of ovarian extracts, and that the cessation of treatment is followed by polyuria and a fall in body weight. Friedlander *et al.* [1936], again, have found that the blood volume of women who have been completely ovariectomized is from 20 to 25 p.c. less than normal, and they have also found that the normal value can be restored by œstrin. These findings can be immediately related to the data recorded in the present paper; further investigation is however necessary to reveal the nature and mechanism of "menstrual œdema" in both women and monkeys.

Summary

1. An investigation was made of the water metabolism of a fully mature female pig-tailed macaque (*Macaca nemestrina*) whose menstrual cycles varied slightly around a mean of approximately 30 days. The

sexual skin of the animal regularly began to swell during menstruation, resorption of the swelling beginning, as a rule, shortly before the middle of the cycle.

2. The animal's body weight fluctuated in accordance with the state of the sexual skin, increasing throughout the first half and decreasing in the second half of the cycle. The onset of the mid-interval decline in body weight agreed to within 24 hours with the onset of sexual-skin subsidence as determined by direct observation. The increase in weight amounted in one cycle to as much as 18 p.c. of the animal's body weight when her sexual skin was quiescent.

3. The mean daily intake of fluid, both in the form of free water and as water provided by the food, was somewhat lower during the phase of sexual-skin swelling than during the phase of sexual-skin subsidence.

4. The amount of urine excreted was much higher during the phase of sexual-skin subsidence than in that of swelling. In each of the three observed cycles a dramatic rise (up to 645 p.c.) in the amount of urine excreted occurred within 24 hours of the observed beginning of sexual-skin subsidence.[1]

5. The amount of water retained by the animal during the phase of swelling agreed closely with the observed increase in body weight.

6. These findings are discussed in relation to observations of the times of excretion of œstrogenic substances in the menstrual cycle.

We wish to express our best thanks to Dr R. B. Fisher for several suggestions and for the help he generously gave us in certain calculations. The animal used in this study was bought and kept with the aid of a grant to S. Z. from the Eugenics Society.

[1] The urine of the monkey that formed the subject of this study was measured daily during her 12th, 13th, 14th and 15th cycles. During this period the animal's total water intake was neither controlled nor measured. The urine she excreted fluctuated in precisely the same way as it did during her 8th, 9th and 10th cycles. As before, the amount of urine on the day following the onset of sexual-skin subsidence was much higher than it was during the previous days (increases of 292 p.c., 244 p.c., 363 p.c. and 218 p.c. respectively).

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"SENSITIZATION" BY INJURY OF THE CUTANEOUS NERVE ENDINGS IN THE FROG

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(*Received* 8 *August* 1936)

The present investigation deals with the sensory discharge in the cutaneous nerves of the frog. Its object was to gain further information about the nature of the end-organs and the nerve fibres which give rise to pain. In the frog the pain fibres are generally supposed to belong to the slow group, conducting at 1·5–4·5 m. per sec., for impulses in these fibres are not set up by tactile stimuli, but only by those which would be likely to damage the skin [Adrian, 1931]. The evolution of the discharge resembles the evolution of the pain sensation in man when the skin is injured; for instance, Hogg [1935] finds a long latency and a slow rise and decline in the impulse frequency. But there is another feature of the pain mechanism in human skin which might be looked for in the impulse discharge, namely the lowering of the threshold of the endings to mechanical stimulation after an injury. Lewis & Hess [1933] have studied the "susceptible state" which develops in the human pain endings after injury, and attribute it to the action of a substance liberated from the injured tissues. We have looked for a similar condition produced in the frog's skin by injury, in the hope that, if it were found, it might be possible to identify the substance responsible for the "susceptible state" in this animal. As will be seen, we have found that injury does lower the threshold of the endings of the slow fibres to mechanical stimulation, but we have been unable to identify a substance as the causative factor. In the course of the investigation we have studied the effects of solutions containing various ions in abnormal amount: some of these will be discussed, as they throw additional light on the differences between the endings of the slow and fast fibres.

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Method

Dorsal cutaneous skin nerve preparations were used from both *Rana temporaria* (English) and *Rana esculenta* (Hungarian) frogs. At times, to preserve the cutaneous blood supply, the skin was left attached to the frog. The preparations were either spread out smoothly over a flat ebonite surface through a slit in which the nerve extended to the electrodes in a moist chamber, or were stretched out on the floor of a wax chamber, the nerve leading to the electrodes through a small hole in the floor. The electrodes consisted of cotton wool dipping into U-tubes of Ringer's fluid, which were connected by silver wires, coated with silver chloride, to the input of the amplifying system. This was of the usual type, with recording by loud-speaker, Matthews' oscillograph, viewing screen and camera.

Pressure was applied to the skin by means of a lever which could be loaded with different weights. The stimulating rod which came in contact with the skin was 3 mm. in diameter and its impact was controlled by an oil dashpot. Injury to the skin was produced by scraping or pressure as described later. Tissue "juice" was obtained by scraping the skin of the frog's legs with a scalpel, the moist scrapings being collected on a small piece of filter paper. Solutions used were:

(1) Ringer: NaCl 0·65 p.c.; $NaHCO_3$ 0·015 p.c.; KCl 0·02 p.c.; $CaCl_2$ 0·025 p.c.

(2) KCl solutions: Ringer with KCl content raised to 0·2 p.c.

(3) $CaCl_2$ solutions: Ringer with $CaCl_2$ content raised to 0·05–0·25 p.c.

(4) NaCl solutions: Ringer with NaCl raised to 1·3, 1·62 and 1·95 p.c.

(5) Glucose solutions: Ringer containing 3–10 p.c. $C_6H_{12}O_6$.

Results

Sensitization by injury

In assessing the effects of injury on the cutaneous nerve endings, the most important factor is to ensure that the same endings are exposed to the same intensity of stimulation, before and after the skin has been injured. To comply with this requirement as nearly as possible, we have used a small glass stimulating rod, attached to a lever which could be loaded with varying weights. The stimulating surface of the glass rod was 3 mm. in diameter and could be applied to a selected spot on the skin and the resulting discharge to a fixed pressure observed. The rod could then be withdrawn without moving the position of the lever, so that after

injuring the skin the rod, on release, would come to rest again with the fixed degree of pressure on the same spot as before.

The skin was injured by very gently scraping the surface with a scalpel in the region where the rod had been resting. This type of injury abolishes or diminishes the response of the tactile system (fast impulses), and if not too severe leaves the slow impulse system intact [Adrian, 1931; Feng, 1933]. Injury was also applied by temporarily increasing the pressure, through the stimulating rod, to 30–50 g. or more. This type of injury also abolishes the fast impulse response, but leaves the slow one unimpaired if the pressure is not too great. The pressure was usually applied interruptedly by withdrawing and releasing the stimulating rod

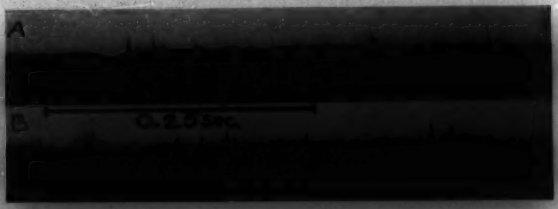

Fig. 1. Slow impulse response to 20 g. pressure applied to the same spot on the skin for 5 sec. A before and B after injury of the skin by gentle scraping. Note that the frequency of the slow impulses is increased after injury.

every 4–5 sec. while observing the discharge in the viewing screen. This procedure gave an opportunity of testing the impulse response at frequent intervals.

Our preliminary experiments showed an apparent increase in the slow impulse discharge after injury. A series of observations was accordingly made with the conditions standardized as far as possible.

Pressures of 10, 15 g., or more, were applied to a selected spot in the receptive field of the skin and the impulse discharge photographed at intervals. After injuring the skin in the manner described, the impulse responses from similar pressures applied to the same area were photographed at corresponding intervals. Some increase in the slow impulse discharge to a given pressure was found in twenty preparations, but in about as many others no definite increase was present, and in a few preparations it was diminished. The increase was never great, and in those preparations regarded as showing it, the discharge, after injury, had a frequency of 1½–4 times that in the uninjured skin (see Fig. 1). In the majority of this group the frequency was doubled after injury.

Although these experiments showed some increase in the response of the slow endings, it was clear that, for proof of a lowering of their threshold, test stimuli of just subthreshold value for the slow endings would have to be used in the uninjured skin; for pressures of 10 g. and over which had been employed were well above the slow impulse threshold.

In a second series of experiments, therefore, the threshold was found in the uninjured skin by observing the discharge in the viewing screen. This varied slightly in different preparations and in different areas in the same preparation, and was usually considerably below 2·0 g. per sq. mm., the value given by Hogg [1935].[1] The just subthreshold degree of pressure for a given area was then determined as nearly as possible and used as the test stimulus. The skin was injured and the same procedure followed as

TABLE I. Frequency of slow impulse discharge per sec.

Prep.	Duration of pressure	Before injury	After injury	Prep.	Duration of pressure	Before injury	After injury
1	Onset	3	5	10	Onset	0	5
	1 sec.	4	8		1 sec.	4	8
	5 ,,	3	10		3 ,,	3	11
	15 ,,	2	8	11	Onset	2	7
2	Onset	0	8		5 sec.	3	15
	1 sec.	1	15		15 ,,	1	15
	5 ,,	0	13	12	Onset	0	4
	15 ,,	1	14		2 sec.	2	8
3	Onset	0	5		5 ,,	1	12
	1 sec.	3	8	13	Onset	3	3
	2 ,,	3	10		5 sec.	3	7
4	Onset	2	8		15 ,,	1	6
	1 sec.	2	11	14	2 ,,	0	10
	5 ,,	5	14		5 ,,	0	9
5	Onset	0	2		15 ,,	0	11
	1 sec.	0	4	15	5 ,,	5	12
	5 ,,	0	8	16	5 ,,	5	20
6	Onset	0	1	17	4 ,,	3	10
	1 sec.	1	2	18	5 ,,	3	19
	3 ,,	0	11	19	5 ,,	4	22
	5 ,,	0	8	20	5 ,,	4	13
7	Onset	1	13	21	5 ,,	3	10
	1 sec.	3	17	22	5 ,,	6	10
	5 ,,	5	17	23	5 ,,	0	14
	15 ,,	3	16	24	5 ,,	4	12
8	Onset	2	8	25	5 ,,	3	20
	2 sec.	4	12	26	5 ,,	5	22
	5 ,,	7	30				
9	Onset	3	9				
	5 sec.	5	16				

[1] In fact values as low as 0·25 g. per sq. mm. have been obtained. This marked variance in the threshold value found by Hogg and ourselves may be due to the difference in the surface area of the stimulating objects used. We have not determined accurately the threshold of the fast fibre endings but they respond to the touch of a fine hair.

already described, photographs being taken of the resulting discharge to the test stimulus at given intervals before and after injury. After injury time was allowed for the spontaneous slow impulse discharge, when present, to subside, and a photograph of the base-line was taken to confirm this, before again photographing the response to the test stimulus.

In every preparation (30) in this series the test stimulus before injury produced only an occasional slow impulse, or none at all, whereas after

Fig. 2. Showing the effect of injury on the response of the cutaneous nerve endings to a constant pressure of 2 g. A, B, C, before and D, E, F, after injury of the skin by gentle scraping. A, at onset of pressure; B, after pressure applied for 2 sec. C, pressure applied for 5 sec. D, E, F, at onset of pressure to same area, and at 2 and 5 sec. respectively. Note that after injury (D, E, F) the slow impulse response is increased and the fast diminished. The latent period to maximum frequency of the slow impulse discharge is evident, being highest in plate F at 5 sec.

injury the discharge was abundant, considering the small degree of pressure used and the usual low frequency in the slow fibres (see Table I and Figs. 2, 3, 4). At the same time, injury temporarily diminished the fast impulse reponse and if severe enough abolished it.

In certain areas of a few preparations, although the test stimulus gave rise to fast impulses, very few slow ones were obtained either before or after injury. In other areas of these preparations, however, the usual "sensitization" of the slow endings resulted from injury. This discrepancy

could easily be explained on the basis of an uneven distribution of the slow endings in the skin. Also in a few areas in several preparations although the test stimulus produced a few slow impulses, no increase was found to the same stimulus after injury. We believe that these latter results can be attributed to too severe injury putting permanently out of action some of the endings. When less injury was used it frequently

Fig. 3. Demonstrates that the threshold to pressure of the slow fibre endings is lowered by injury. A, response from uninjured skin subjected to a pressure of 2 g. for 5 sec., showing only fast impulses. B, response from same area to 2 g. pressure for 5 sec., after injury of the skin by scraping. Abundant slow impulses are now present.

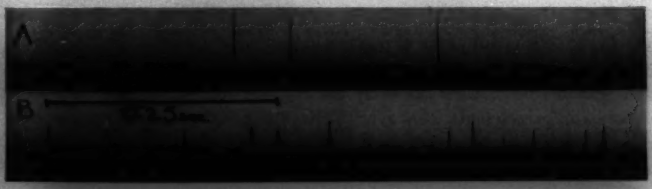

Fig. 4. Impulse response to 2 g. pressure applied to the same spot on the skin for 15 sec. A, before and B, after injury of the skin by gentle scraping. Demonstrates that the threshold to pressure of the slow fibre endings is lowered by injury, since slow impulses are present only in B.

happened that only a feeble discharge of slow impulses was obtained until several minutes had elapsed, when it became abundant. This interval, although it could be the result of the gradual action of some "sensitizing" substance, has seemed more likely to be due to the slow recovery of the endings from trauma since it is not usually evident if the injury is still less severe. These latter effects, as well as "sensitization" of the slow endings, have been more easy to demonstrate in Hungarian than in English frogs, since injury is less likely to destroy their nerve endings.

As Hogg found, there is a considerable latent period after pressure is applied before the frequency of the slow impulse discharge reaches its maximum [Hogg, 1935]. With the lighter pressures used (under 2·5 g.) this latency has been as long as 5 sec., but with heavier pressures (15–20 g.) it has usually been about 0·75 sec. [Hogg, 1935]. We have not, however, found any appreciable alteration in the length of this latent period to a given pressure before and after injury although, of course, the frequencies are different.

Although the frequencies are higher after injury it is of interest that the highest frequency of slow impulse discharge we have obtained with mechanical stimulation over a surface of 3 mm. in diameter was 60 per sec., even when several units were in action. According to Hogg, 40 per sec. is the maximum rate at which single units can discharge by themselves. As to the adaptation time of these ending we agree with Hogg that it is extremely slow, for on several occasions after injury a pressure of 15 g. has given rise to a discharge of slow impulses, after the first ½ min. that showed little evidence of diminished frequency in half an hour. We also agree with him that the slow impulse discharge "never ceases abruptly on cessation of stimulation", but we find that often only a few scattered impulses occur after withdrawal of the stimulus.

During the course of these experiments it was observed that the discharge of slow impulses to a given pressure for a fixed duration varied widely in frogs of the same type at different periods. For instance, in Hungarian frogs during the early part of the breeding season, and in a large batch which had ulcers over the head regions, the discharge reached 40 per sec. with 15 g. pressure in the uninjured preparation. This is about double the frequency obtained in the same type of frogs during other periods.

The effect of potassium on the cutaneous nerve endings

Feng [1933] has considered the possibility that a liberation of potassium might account for some of the sensory effects of injury. He showed that the temporary abolition of the tactile response when the frog's skin is scraped might be due to an excess of K ions; for he found that a reversible abolition of tactile impulses could be produced, without apparent effect on the slow fibre system, by placing injured tissues on the subcutaneous surface of the skin, or exposing it to a fluid containing the concentration of potassium which would be present in the injured tissue (i.e. about ten times the amount in normal Ringer's fluid).

Although Feng had considered and rejected the suggestion that the excess of K might stimulate the slow endings, it seemed possible that it might play some part in their sensitization to mechanical stimulation.

From Feng's account it was not certain that the site of action of the excess potassium was on the nerve endings rather than on the nerve fibres, since the K solutions or tissue scrapings were placed on the subcutaneous side of the skin, where they might come in contact with the nerve. It seemed possible therefore that the slow impulses which can be obtained after treatment of a preparation with potassium solution might be fast ones travelling in a nerve whose conduction had been slowed. We find, however, that 0·2 p.c. K solutions though they abolish the tactile response from the area of skin to which they are applied are without definite effect on the nerve fibres in the cutaneous nerve. The evidence for this comes from experiments in which the nerve was slung across an electrode in which the cotton wool was soaked in Ringer's fluid, containing 0·2 p.c. K (ten times the normal amount). In no case after an interval of 20 min. was there any interference with the conduction of the fast impulses past the electrodes, though the same solution applied to the back of the skin abolished the fast impulses in 5–15 min. Also, when the potassium was restricted to a small area of the back (subcutaneous surface) of the skin, by placing it on swabs of cotton wool, it abolished the fast impulse response in this area, but not from the neighbouring regions. The nerve therefore was conducting normally and the slow impulses which were easily obtained from the area treated with K were genuine ones, and not fast impulses travelling in a slowly conducting nerve. It was further observed that the elevated touch spots were the last to lose their response to touch when the K solution was applied to the whole preparation or to the subcutaneous surface alone. This was often found even when the touch spots were widely scattered over the skin, the intervening areas becoming unresponsive. The reason why they should be affected later than the other endings is possibly that the K would take longer to reach them, since the K, to have effect, must be applied to the subcutaneous surface of the preparation. 0·2 p.c. K solutions restricted to the outer surface of the preparations, where the elevated touch spots are located, had no effect in half an hour. We conclude that the action of the excess K is on the nerve endings, or at any rate on the terminal parts of the nerve fibres rather than on the nerve trunks.

We have confirmed Feng's finding that injured tissue (the scraping of skin from frog's legs) applied to the subcutaneous surface of the skin acts like K solutions in abolishing the tactile response. We have also found, as he did, that neither injured tissue nor K solutions give rise to a spontaneous discharge of slow impulses from the skin. Occasionally a few have appeared, but they were most likely the result of handling the preparation.

On the question of the sensitization of the slow fibre endings, however, we have found it very difficult to obtain conclusive evidence. With pressures of 2 g. per sq. mm., and above, slow impulses are easy to obtain in a preparation treated with potassium, and in a few cases there has been a slight increase in impulse frequency over the untreated preparations. The increase is certainly not as great as with injury however, and we are not convinced that it is outside the range of error. Several difficulties arise in interpreting the results. In the first place, since pressure must be

used to obtain the slow impulses, any increase in the discharge that is observed may be due to the injury produced by the pressure itself. The results are complicated as well by the fact that a solution containing 0·2 p.c. K will abolish even the response of the slow system in about half an hour, so that a sensitizing effect, if its exists, will soon merge into a depression. The preparations in which all response has been abolished in this manner show a recovery of both slow and fast impulse response after an immersion for an hour in normal Ringer's fluid.

Stimulation of the endings of the slow and fast fibres by hypertonic solutions and $CaCl_2$

Although injury is not likely to cause an increase in the osmotic pressure of the tissue fluids sufficient to stimulate the pain endings, it is well known that subcutaneous injections of hypertonic glucose or NaCl

Fig. 5. Shows the impulse response after irrigating the skin with Ringer's fluid containing $2\frac{1}{2}$ × the normal quantity of NaCl. A, response when skin irrigated for 1 min. and is composed entirely of slow impulses. B, spontaneous rhythmical outburst of fast impulses which occurred after 20 min. irrigation.

solutions will cause pain. We have therefore investigated the effects of 4–10 p.c. glucose and of 1·3–1·95 p.c. NaCl solutions on the frog's skin.

Preparations irrigated or immersed in Ringer's fluid containing 4–10 p.c. glucose gave a persistent spontaneous discharge of slow impulses, which came on within $\frac{1}{2}$–2 min. and showed only a slightly diminished frequency during the following hour. Fast impulses appeared after about 5 min., but remained infrequent except with 7·5–10 p.c. glucose solutions. Even with these concentrations the fast impulse discharge remained irregular except for a few short rhythmical bursts.

In preparations treated with NaCl 1·3–1·95 p.c. Ringer's fluid, however, although they showed the same type of spontaneous slow impulse discharge (see Fig. 5A), long rhythmical discharges of fast impulses appeared on touching the skin with a glass rod in 15–20 min. after irrigation (see Fig. 5B). Since the tendency to rhythmical impulse discharges

is much less marked in preparations treated with glucose solutions of the same osmotic pressure, this effect of the NaCl is evidently due to the disturbance of the Na/Ca balance. Talaat [1933] found a similar type of rhythmical after-discharge in preparations bathed in Ca free Ringer's solution (0·65 p.c. NaCl) and this would disappear if Ca were added to the irrigating fluid.

Since Talaat [1933] also found that removing the Ca from the Ringer's fluid bathing a preparation would cause a lengthening in the adaptation time of the sensory receptors, it seemed interesting to try the effect of increasing the calcium content of the Ringer's fluid above the normal

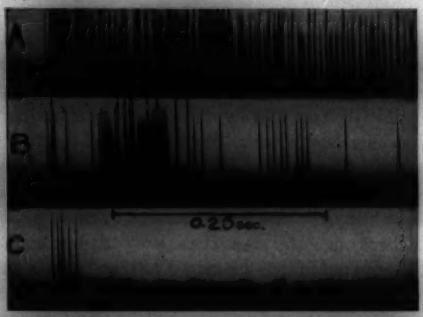

Fig. 6. Shows the impulse response to 10 g. pressure on the skin before and after irrigation with Ringer's fluid containing 0·25 p.c. $CaCl_2$. A, response to pressure in normal skin. B, response to pressure over an elevated touch spot after 10 min. irrigation. C, response to pressure over the smooth portion of the skin, taken just before B.

value. Frogs, during the breeding season and for a considerable period after, are very suitable for a study of this kind, as the endings of the fast fibres adapt quite slowly to a constant pressure. For instance, a constant pressure of 10 g. causes an outburst of fast impulses, which maintains a high but diminishing frequency for from 5 to 15 sec., thereafter becoming irregular and diminishing rapidly in rate. This type of discharge was photographed in normal preparations (see Fig. 6A) and after immersing the preparations in or irrigating their subcutaneous surface with 0·05–0·25 p.c. $CaCl_2$ (twice to ten times the normal amount in Ringer's fluid). The most obvious result of the treatment was that the receptors of the fast fibres became much more rapidly adapted to the pressure: this is in agreement with Matthews' finding [1931] that if $CaCl_2$ is added to a

NaCl solution bathing a frog's muscle spindle the adaptation time to stretch becomes shortened. After irrigation with 0·25 p.c. $CaCl_2$ (ten times the amount in normal Ringer's fluid) for 10 min., although the preparations' sensitivity to light touch was not obviously diminished, the discharge to pressure was reduced to one that was practically over in less than a second (see Fig. 6C). A similar effect was obtained with 0·05 p.c. $CaCl_2$ chloride, but it took much longer to be established, the preparations usually being immersed in the fluid and placed in the ice box for several hours. With the higher concentrations the effect of the calcium was easily restricted to a small area of the preparation as described with potassium. Again, the last areas to show a shortening of discharge were the elevated touch spots. This was quite striking, for often pressure anywhere gave only a momentary outburst of impulses until, coming in contact with a touch spot, a discharge of considerable duration occurred (see Fig. 6B). Apart from a shortening of the discharge the $CaCl_2$ solutions also diminished the initial frequency and, in the higher concentrations, if allowed to act long enough, abolished the fast impulses. After these had gone, pressure still produced a discharge in the slow fibres, which did not appear shorter than in the normal preparation. In time, however, the slow impulse response was also abolished by the calcium solution if used in the higher concentrations. These preparations usually recovered if placed in Ringer's fluid for 1–2 hours. $CaCl_2$ up to 0·25 p.c. in Ringer's solution, like K, never produced a spontaneous discharge, and if placed on the outer surface of the preparation had no effect on the response to pressure. Replacing the Ringer's fluid in the first electrode with Ringer's containing 0·25 p.c. $CaCl_2$, and leaving the nerve in contact with this for 20 min., had no apparent effect on the discharge from stimulating the skin.

In Hungarian frogs a peculiar effect was noted, and it is here mentioned so that others may not be misled, as we were at first. If a moist material of apparently any nature is placed on the surface of the skin of these frogs a spontaneous discharge of fast impulses results. It occurs with cotton wool soaked in water, Ringer's fluid, Ringer's fluid plus 0·2 p.c. K, or 0·25 p.c. $CaCl_2$, as well as with tissue scrapings from the frog's skin. The spontaneous discharge comes on slowly, a few fast impulses occurring only after about 5 min. These gradually increase in frequency, reaching their maximum (94 per sec.) at 15–20 min. but always remaining irregular. Only a very few slow impulses appear, but the fast continue undiminished as long as the stimulating material is present. When it is removed the discharge subsides only gradually, taking 2–5 min. to disappear. The

preparation is then found to be normally sensitive even after an hour of this treatment. The same phenomena were observed when the skin from these frogs was immersed in Ringer's fluid (in a wax chamber). From the nature of the discharge it seems probable that it is due to some mechanical disturbance, but we are unable to explain it.

Discussion

It has been found that the endings of the slow conducting fibres in the frog's skin show a lowering of their threshold or an increase in response to mechanical stimulation following injury. Human pain endings also become "hypersensitive" after injury, but this is no proof that their function is in any way similar to the endings of the slow fibres in the frog. The finding is, however, not out of keeping with the supposition that the frog's slow fibre system is concerned with pain, and if the supposition is correct it suggests another point of similarity between the frog's and the human pain mechanism.

It is interesting to consider together the effect on the frog's skin of injury, tissue scrapings and potassium. Either one will abolish the response of the fast impulses (response to light touch) before the slow ones (response to pressure), and, as Feng has shown, it is apparently potassium that is responsible in the tissue scrapings. Again, if one examines the effect of injury on the human skin by pressing a hard object (the wooden end of a match) firmly against the back of the hand for several minutes until pain results, one finds that the sensation to light touch has become diminished in the area pressed upon. Whether the mechanism is similar in the two cases and whether potassium is responsible we are unable to say.

With regard to the role of ions in stimulating the endings of the slow fibres to discharge, there is no evidence that this occurs under ordinary circumstances in the frog. The sodium calcium ratio probably plays a role in normal adaptation but it is unlikely that the calcium would ever be reduced or the sodium raised sufficiently to cause an impulse discharge. There is still the possibility that potassium is concerned in the "sensitizing" of the slow fibre endings by injury, but we have been unable to find conclusive evidence of this.

Summary and conclusions

1. The effect of injury, potassium, calcium and hypertonic sodium chloride and glucose on the cutaneous nerve endings in the frog have been studied.

2. Pressure on the frog's skin, like scraping, has a differential action on the various nerve endings, abolishing the response of the receptors for light touch (fast impulses) before those responding to pressure (slow impulses).

3. Injury to the frog's skin either by pressure or gentle scraping causes a lowering of the threshold or an increase in response to mechanical stimulation of the endings of the slow conducting fibres.

4. These endings, like the pain endings in the human skin, are therefore "hypersensitive" following injury.

5. The action of potassium chloride in abolishing the response to touch in the frog's skin is on the terminal nerve fibres or nerve endings.

6. The touch receptors in the frog's skin adapt more rapidly after treatment with calcium chloride and its action is on the terminal nerve fibres or nerve endings.

We are deeply grateful to Prof. Adrian for his guidance during the investigation and his aid in preparing this paper; and to Mr B. H. C. Matthews for his constant advice.

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THE RELATION OF THE PITUITARY TO LIVER GLYCOGEN PRODUCTION AND UTILIZATION

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(*Received* 10 *August* 1936)

The classical studies of Houssay have demonstrated an intimate relationship between the anterior pituitary and carbohydrate metabolism. These findings have been confirmed and expanded by work in several laboratories. The mode of action of the pituitary beyond its antagonism to insulin still remains obscure. Experiments now to be reported were undertaken in an attempt to elucidate what part, if any, the liver glycogen played in relation to the pituitary.

Houssay and his collaborators [1933], and Corkill *et al.* [1933], have shown that the insulin sensitivity of the hypophysectomized animal exists in spite of normal amounts of liver glycogen. Houssay *et al.* [1933] have shown in the toad that a slow fall occurs over several days in both the liver and muscle glycogen. A parallel fall in blood sugar was not demonstrated. In hypophysectomized dogs Houssay [1936] has shown a diminished quantity of liver glycogen as compared to controls, but the amounts are within normal limits. Corkill *et al.* [1933], working with adult hypophysectomized rabbits, found that the animal dying in insulin hypoglycæmia had normal amounts of liver glycogen. In those animals dying in spontaneous hypoglycæmia there was "a greatly depleted liver glycogen content". In a single experiment with two hypophysectomized baby rabbits these workers observed more liver glycogen in the animal injected with insulin than in the control. Since they failed to determine the blood-sugar level in either animal, in view of the experiments recorded in this paper, no conclusion is possible. Other experiments in relation to liver glycogen and the whole pituitary gland have been recorded by Phillips & Robb [1934], who found diminished glycogen deposition

from glucose given by stomach tube to hypophysectomized rats. It is possible that the diminished glycogen deposition was not due to an alteration in hepatic function but to diminished intestinal absorption.

The baby rabbit was chosen for the present experiments since it has been shown by Goldblatt, and fully confirmed by Corkill, that the normal baby rabbit, unlike the rat or mouse, deposits liver glycogen following the injection of insulin or small amounts of adrenaline. Because of the apparent antagonism between insulin and the anterior pituitary it was thought that this animal might elucidate to a certain degree any function the pituitary had which would be obscure in some other animal.

Methods

Rabbits 6–8 weeks old, weighing from 600 to 1000 g., were used. Hypophysectomy was performed through the nasopharynx and base of the sphenoid bone. By this approach brain trauma is eliminated except for the traction of removing the stalk. No attempt has been made to differentiate between anterior and posterior lobe. The hypophysectomy includes both major lobes, the pars tuberalis and the stalk, and is, therefore, total. In all the earlier and subsequent crucial experiments the completeness of hypophysectomy has been proved by serial section of the base of the brain as well as the contents of the sella turcica. Control operations were performed going through the entire procedure, except the actual removal of the pituitary, in order to exclude operation as a possible factor. To avoid spontaneous hypoglycæmia it has been found necessary to maintain the animals on stomach tube feedings of glucose and milk given twice daily during the interval between operation and experimental observations. Lettuce, carrots and rolled oats were available to the animals but were eaten only spasmodically. The animals were sacrificed by decapitation. The blood sugar was determined by the micro method of Folin [1932]. The liver and muscle glycogen were determined in duplicate by the Lovatt Evans, Tsai, and Young modification [1931] of the original Pflüger method.

Control observations

Control observations were first made to study the relation of the blood-sugar level to the amount of liver glycogen in the untreated hypophysectomized animal. The experiments have been conducted in the following manner. The day before the experimental observations all food except water is removed. At 9 a.m. of that day the animal is given its

routine stomach tube feeding of 12–15 c.c. of 50 p.c. glucose in saline and 8–10 c.c. of milk. At 9 p.m. a feeding of 50 p.c. glucose alone is given in order to eliminate protein, fat and the higher carbohydrates, all of which require digestion and are followed by a more prolonged period of absorption. The following morning at 9 a.m. hourly blood-sugar determinations are started.

The first animal on Chart 1, No. 190, shows blood-sugar observations made from 9 a.m. until 2.30 p.m., when it was sacrificed. During the period of observation there was a slow decline in the blood sugar. The

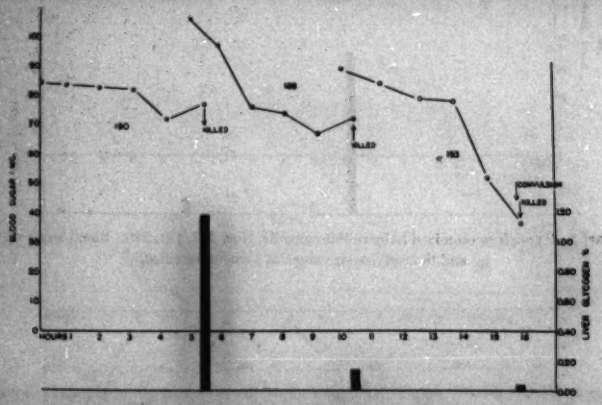

Chart 1. Hypophysectomized baby rabbit controls, Nos. 190, 188, 193. Blood-sugar curves and liver-glycogen values at time of sacrifice.

liver glycogen found at death, 1·18 p.c., is well within limits for a normal animal during digestion. The second animal, No. 188, observed over the same period of time following a similar stomach tube feeding, showed in the first 2 hours a rapid decline in the blood sugar until a level of 75 mg. was reached, after which the blood sugar fell very slowly during the next 3½ hours. When it was sacrificed the liver glycogen was at the lower level of the expected average level of the fasting normal animal. The third animal on Chart 1, No. 193, observed slightly longer than the previous two animals, showed for the first 4 hours a slow decline in the blood sugar, changing abruptly to a rapid decline to convulsive levels. When sacrificed immediately following a convulsion the liver glycogen was present only in traces.

Chart 2 shows the blood-sugar curves and liver-glycogen values of three very similar animals. It will be noted that in the first two the blood sugar

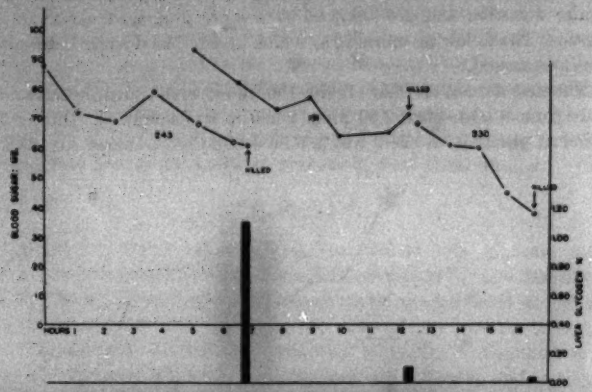

Chart 2. Hypophysectomized baby rabbit controls, Nos. 243, 191, 230. Blood-sugar curves and liver-glycogen values at time of sacrifice.

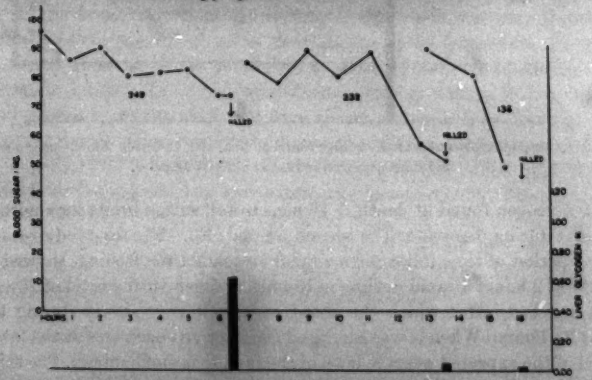

Chart 3. Hypophysectomized baby rabbit controls, Nos. 249, 232, 136. Blood-sugar curves and liver-glycogen values at time of sacrifice.

did not go below 60 mg., and in the levels between 80 and 60 mg. the decline was slow. At death glycogen values were within normal limits.

The third animal however, No. 230, had a rapid decline in blood sugar below 60 mg. and had but a trace of liver glycogen at death.

Chart 3 shows another series of three animals; the first, No. 249, is similar to the first on the other charts. The second animal, No. 232, maintained a plateau at 80 mg. for 5 hours, followed by an abrupt fall. Only a trace of liver glycogen was found at death. The last animal, No. 136, was observed only during a rapid decline and was found to have but a slight trace of liver glycogen.

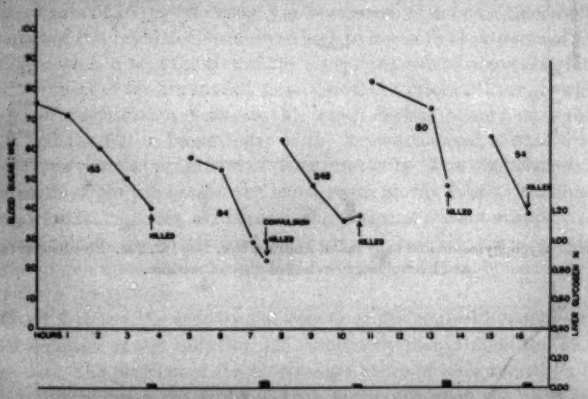

Chart 4. Hypophysectomized baby rabbit controls, Nos. 153, 84, 248, 50, 54. Blood-sugar curves and liver-glycogen values at time of sacrifice.

Chart 4 pictures five more animals all showing an abrupt fall in blood sugar from the region of 60–80 mg. down to convulsive levels, with but traces of liver glycogen at death.

In Chart 5 the first animal, No. 246, shows a plateau at the unusually low level of 60 mg. The other two, Nos. 192 and 238, show no decline below the plateau and yet had but traces of liver glycogen when sacrificed. It is suggested that had these latter two experiments been continued an hour longer, an abrupt decline in the blood sugar would have been apparent.

Chart 6 shows three exceptions to the experiments described above. The first and third animals showed rapid declines in blood sugar in the presence of amounts of liver glycogen consistent with the normal fasting animal. Animal No. 195 showed a slow fall in blood sugar, but at levels

just above convulsions. At death a normal fasting amount of liver glycogen was found. Serial sections revealed complete hypophysectomy

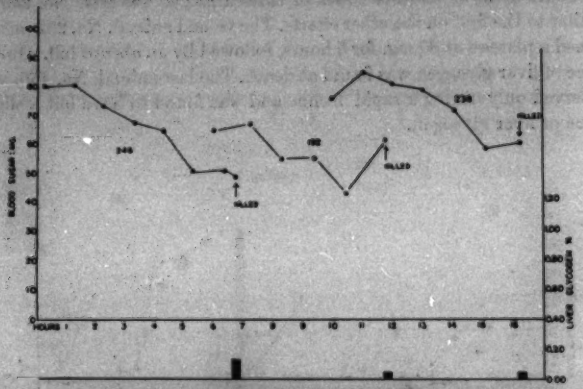

Chart 5. Hypophysectomized baby rabbit controls, Nos. 246, 192, 238. Blood-sugar curves and liver-glycogen values at time of sacrifice.

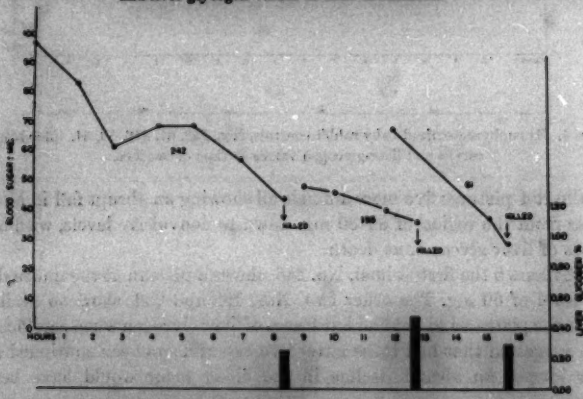

Chart 6. Hypophysectomized baby rabbit controls, Nos. 242, 195, 61. Blood-sugar curves and liver-glycogen values at time of sacrifice.

which would be expected, since a rabbit with intact residual pituitary tissue does not exhibit spontaneously such low blood sugars. These

experiences have not been seen in any of the other hypophysectomized animals. They are presented in contrast.

A study of the blood-sugar curve in fifty-six hypophysectomized animals has shown that following the glucose feeding the blood sugar is maintained within normal limits; as absorption ceases the blood sugar gradually declines to a level of 80–60 mg. At this subnormal level a slightly inclined plateau is maintained for from 2 to 6 hours when an abrupt change occurs and the blood sugar falls rapidly to convulsive levels. This rapid preconvulsive drop in blood sugar occurs at a nearly constant rate of 15 mg. per hour. The seven control animals killed before the onset of this rapid decline showed high to low normal amounts of liver glycogen, while nine, exclusive of the exceptions quoted above, sacrificed after the onset of the abrupt fall, have shown but traces of liver glycogen. The close resemblance between this rapid decline toward convulsive levels and the fall in blood sugar of the hepatectomized animal is evident [Drury & Salter, 1934]. It is suggested by these studies: one, as absorption of glucose ceases, the blood sugar slowly falls to subnormal levels below 80 mg.; two, as long as liver glycogen is present in appreciable amounts this decline of the blood sugar continues slowly; three, when the liver-glycogen store becomes depleted the rapid fall in blood sugar to convulsive levels occurs.

Chart 7 shows the blood-sugar curves of the control hypophysectomized animals, except only No. 195, arbitrarily placed upon the blood-sugar mat. The position of the blood-sugar curve of each animal on this mat depended upon the trend of that curve, not upon the number of hours since the last feeding. Differences in weight of the animals, with consequent differences in total metabolism, gave greater variations in time from feeding to convulsions than the duration of the terminal rapid decline in blood sugar. The curves on the first two charts were plotted on Chart 7 in approximately the same position as they are on Charts 1 and 2. All the curves in Charts 3–6 showing rapid declines were placed in the later hours of Chart 7. Along the rough curve so constructed all the remaining individual blood-sugar curves were plotted in appropriate places. A line was then drawn through the points on the mat constructing a composite curve. The liver glycogens are placed beneath the terminal blood-sugar value of each animal. It will be noted that the blood-sugar level is maintained above 60 mg. as long as liver glycogen is present in appreciable quantities, and with one exception (No. 61), when only traces of liver glycogen are present, the blood sugar is in rapid decline toward convulsive levels.

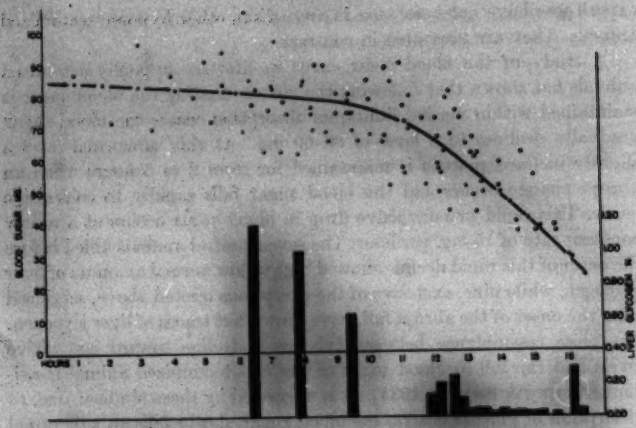

Chart 7. Composite chart of blood-sugar curves and liver-glycogen values of all baby rabbits shown in Charts 1–6 except No. 195.

TABLE I. Hypophysectomised controls

No.	Days after operation	Blood sugar mg./100 c.c.	Blood lactate mg./100 c.c.	Convulsion	Glycogen p.c. Liver	Glycogen p.c. Muscle
(a) With blood sugar at death of 60 mg. per 100 c.c. or less						
50	4	48	—	0	0·050	0·049
54	4	39	—	0	0·034	0·214
61	6	28	—	0	0·296	0·240
84	7	23	—	+	0·049	0·188
136	4	48	—	0	0·030	0·231
153	4	40	—	0	0·033	0·242
192	7	60	13	0	0·050	0·427
193	7	36	95	+	0·038	0·102
195	8	35	32	0	0·480	0·222
230	6	38	—	0	0·038	0·346
232	5	50	—	0	0·046	0·384
238	6	59	—	0	0·050	0·415
242	6	43	—	0	0·255	0·298
246	6	48	—	0	0·205	0·101
248	6	38	—	0	0·026	0·058
				Average	0·112	0·234
(b) With blood sugar at death above 60 mg. per 100 c.c.						
188	7	71	46	0	0·139	0·401
190	6	76	22	0	1·180	0·216
191	7	71	17	0	0·113	0·233
243	6	61	—	0	1·155	0·453
249	6	73	—	0	0·632	0·165
				Average	0·844	0·294

In Table I this becomes perhaps more apparent. The average value for liver glycogen of fifteen animals with a blood sugar at death of 60 mg. or less is 0·112 p.c., of five animals with a blood sugar above 60 mg. is 0·644 p.c. The table also shows the individual blood-sugar, liver- and muscle-glycogen values.

From these control observations it is apparent that the fasting hypophysectomized rabbit does not just drift into hypoglycæmia. There are two distinct phases to the curve. As long as liver glycogen is present the animal is able to maintain his blood-sugar level. When the store becomes depleted a rapid decline of the blood sugar similar to that seen in the hepatectomized animal occurs until the animal dies in convulsions. The condition of the liver is the final determining factor.

Liver glycogen deposition

Studies of liver glycogen deposition in the baby rabbit by Goldblatt [1933, 1936], Corkill & Ochoa [1934] and Cope & Corkill [1934] have thrown considerable light on the interrelated actions of various hormones in carbohydrate metabolism. The normal baby rather than adult rabbit is used because it has relatively constant fasting liver-glycogen values. The studies in the first portion of this paper show that in the hypophysectomized baby rabbit a similar constancy of liver glycogen exists. Once a rapid decline in blood sugar from 60 mg. has been demonstrated it can be assumed that the liver glycogen value is below 0·1 p.c. In a study of liver-glycogen deposition in the absence of the pituitary each animal, therefore, serves as its own control.

Chart 8 shows a composite graph of the liver-glycogen contents of the unoperated and the hypophysectomized controls and animals following various procedures. The figures for the unoperated animals are from a small series of controls treated approximately in the same manner as the hypophysectomized animals. In the unoperated animal these reactions are well known from previous studies in the literature. The actual amounts of hepatic glycogen vary with the amount of the substance injected and the time between the injection and sacrifice of the animal.

Glucose, injected into the veins, is deposited readily in the normal animal as liver glycogen, and this is apparently true also of the hypophysectomized rabbit (Table II), in which spontaneous hypoglycæmic convulsions are thus quickly arrested. An animal, prostrate between the convulsive seizures, will be standing before the injection is completed if this is started early enough. If the animal is already nearly moribund from prolonged convulsions, however, when the injection is given it may

not recover even though its blood sugar may be normal or above normal. Grayzel [1934] has reported brain damage in rabbits following insulin convulsions, the amount of damage being proportional to the number or

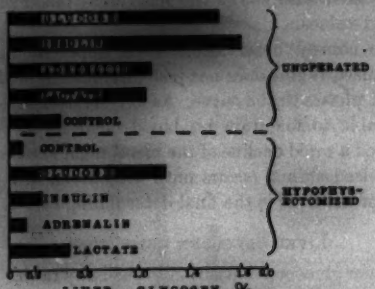

Chart 8. Average liver-glycogen values of unoperated and hypophysectomized baby rabbits.

TABLE II. Intravenous injection of glucose

(a) In unoperated controls after 48 hours' fast

No.	Blood sugar mg./100 c.c. Injection	Death	Glucose c.c. 25 p.c.	Interval min.	Glycogen p.c. Liver	Muscle
102	54	256	12	75	0·589	0·037
106	89	235	12	74	0·902	0·043
166	156	267	12	20	0·956	0·080
167	116	278	10	53	1·112	0·127
168	133	185	10	92	4·780	0·105
171	91	210	12	30	2·450	0·179
172	95	400	12	41	1·290	0·204
173	105	500	12	20	0·850	0·198
				Average	1·616	0·122

(b) In hypophysectomized animals

No.	Blood sugar mg./100 c.c. Injection	Death	Glucose c.c. 25 p.c.	Interval min.	Glycogen p.c. Liver	Muscle	Days after operation
18*	95	183	24	132	3·510	—	4
47	—	133	13	91	1·520	—	3
90	41	200	20	77	2·140	0·220	4
160	—	328	13	68	1·011	0·078	4
194	36	263	13	50	1·280	0·329	8
198	31	290	13	20	0·820	0·211	8
201	28	282	13	40	0·486	0·056	7
				Average	1·210	0·179	

* Omitted from average.
Convulsions occurred in Nos. 47, 90, 160 and 201.

duration of convulsions. No attempt has been made to demonstrate brain damage in this series of animals. The amounts of glycogen found in the liver as shown in Chart 8 suggest a diminished utilization of the glucose in the hypophysectomized rabbit; but it should be recalled that at the start of the injection the blood sugar was lower and the amount of liver glycogen less than in the unoperated animal. There is no evidence that the utilization of intravenous glucose in the hypophysectomized rabbit is diminished.

Lactate. Intravenous *dl*-sodium lactate, like glucose, is deposited as liver glycogen in the normal animal (Table III). Following the injection

TABLE III. Intravenous injection of lactate

(*a*) In unoperated controls after 48 hours fast

No.	Blood sugar mg./100 c.c. Injection	Blood sugar mg./100 c.c. Death	Na lactate c.c. 20 p.c.	Interval min.	Glycogen p.c. Liver	Glycogen p.c. Muscle
100	104	200	8	83	0·412	0·080
107	93	200	8	84	1·120	0·164
129	109	136	8	120	1·650	0·090
162	107	146	7·5	61	1·750	0·026
163	82	122	8	41	0·834	0·090
164	86	114	8	20	0·525	0·195
182	123	148	8	100	1·450	0·148
183	104	174	8	70	1·500	0·144
184	104	141	8	30	1·020	0·095
				Average	1·140	0·115

(*b*) In hypophysectomized animals

No.	Blood sugar mg./100 c.c. Injection	Blood sugar mg./100 c.c. Death	Na lactate c.c. 20 p.c.	Interval min.	Glycogen p.c. Liver	Glycogen p.c. Muscle	Days after operation
19*	85	83	8	120	0·610	—	4
40	—	58	8	91	0·190	—	4
49	52	74	8	100	0·080	0·081	4
56	41	50	13·7	160	0·036	0·033	4
146	47	64	8	60	0·507	0·102	4
151	61	93	8	20	0·037	0·109	3
196	49	56	8	81	1·290	0·217	7
199	38	44	8	40	1·110	0·203	7
				Average	0·464	0·124	

* Omitted from average: preliminary blood sugar not determined. Convulsions occurred in Nos. 40, 56 and 199.

there is a rise in blood sugar. In the hypophysectomized animal the deposition of sodium lactate apparently occurs to a less degree than in the normal animal. The intravenous injection of lactate is followed by a slow rise in blood sugar. When the lactate is injected into an animal in spontaneous hypoglycæmic convulsions, only after 20 min. is the animal

standing and able to walk. Its utilization is much slower than that of glucose. In the normal animal given a convulsive dose of insulin, intravenous lactate is apparently utilized very much more rapidly, but this experiment is difficult to evaluate since the convulsion is in itself such an efficient mechanism for raising the blood sugar.

Adrenaline. Small subcutaneous injections of adrenaline into a normal rabbit cause deposition of liver glycogen. In the hypophysectomized rabbit adrenaline causes little if any deposition of liver glycogen (Table IV).

TABLE IV. Injection of 0·2 mg. adrenaline hydrochloride

(a) In unoperated controls after 48 hours fast

No.	Blood sugar mg./100 c.c. Injection	Death	Interval min.	Glycogen p.c. Liver	Muscle
103	105	148	40	0·428	0·038
108	94	137	40	0·990	0·042
128	93	171	110	1·400	0·086
155	90	160	90	1·890	0·017
178	111	134	20	0·231	0·262
179	109	146	30	0·219	0·162
180	119	171	60	0·317	0·000
			Average	0·782	0·090

(b) In hypophysectomized animals

No.	Blood sugar mg./100 c.c. Injection	Death	Interval min.	Glycogen p.c. Liver	Muscle	Days after operation
57	42	40	40	0·066	0·103	7
60	60	59	40	0·010	0·003	6
82	45	48	25	0·357	0·259	12
85	49	70	20	0·324	0·086	7
87*	74	80	60	1·080	0·334	7
134	53	51	60	0·207	0·070	4
138	43	46	20	0·044	0·111	4
144	52	80	100	0·039	0·086	3
154	71	67	80	0·073	0·210	3
			Average	0·141	0·116	

* Omitted from average. Blood sugar did not fall from 10 a.m. to 1.16 p.m., time of sacrifice.

Convulsions occurred in none of the above animals.

The blood sugar shows a slight rise, the diminished response seen in the hypophysectomized adult rabbit [Corkill *et al.* 1933; Cope & Marks, 1934]. Cope & Thompson [1936] have found a normal rise in blood lactate following adrenaline in the hypophysectomized adult rabbit. It is possible that the failure of the hypophysectomized baby rabbit to deposit glycogen is not due to a lack of available lactate. Although lactate is probably transformed into glycogen and glucose, the rate at which this

occurs may be so diminished that no glycogen is stored in the liver from the relatively small amount of blood lactate available following adrenaline action. Cope & Marks [1934] have suggested that the diminished blood-sugar response to adrenaline observed in the hypophysectomized adult rabbit may be due to the inability of adrenaline to break down the stored liver glycogen into glucose. In baby rabbits the rate at which liver glycogen is discharged as sugar may be determined by the rate at which it is formed from lactate. It is probable that the reaction is complex, involving more hormones than the anterior pituitary secretion and adrenaline.

Insulin, given in doses sufficient to cause considerable hypoglycæmia, is accompanied in the normal rabbit by liver glycogen deposition (Table V). This presumably is caused by the secondary secretion of

TABLE V. Injection of insulin

(a) In unoperated controls after 48 hours fast

No.	Blood sugar mg./100 c.c. Injection	Death	Convulsion	Insulin units	Interval min.	Glycogen p.c. Liver	Muscle
105	83	43	+	0·5 + 0·25	163	2·180	0·036
111	83	53	+	0·5 + 0·75	241	2·780	0·010
131	93	32	0	1·0 + 0·90	235	3·110	0·041
					Average	2·690	0·029

(b) In hypophysectomized animals

No.	Blood sugar mg./100 c.c. Injection	Death	Convulsion	Insulin units	Interval min.	Glycogen p.c. Liver	Muscle	Days after operation
12	65	21	+	0·25	42	0·360	—	5
44	35	23	+	0·10	11	0·037	—	4
48	63	37	+	0·15	23	0·329	0·167	4
53	59	24	+	0·10	24	0·274	0·272	4
141	73	32	0	0·20	45	0·495	0·400	4
150	50	25	0	0·20	13	0·069	0·155	4
					Average	0·261	0·249	

adrenaline in response to hypoglycæmia [Cope & Corkill, 1934]. The experiments with insulin in the hypophysectomized animal cannot be evaluated, since the animal is so sensitive to insulin that after the initial fall in blood sugar has been determined but little time remains for insulin to act before convulsions occur. Of the six animals used in this experiment only two, Nos. 44 and 150, were properly controlled. In each of these a preliminary rapid drop in blood sugar from the plateau level was demonstrated. The liver glycogen values determined were within the limits of the controls. Only a very short time elapsed for the insulin to act. In animal

No. 12 no preliminary blood-sugar curve was determined. The liver-glycogen value found at death is within the range expected of an animal with an initial blood sugar of 65 mg. In rabbits Nos. 48 and 141 the preliminary blood-sugar curve showed the animals to be still in the plateau region. The glycogen values at death are, therefore, within the expected limit at the time of injection. Animal No. 53 is on the border-line. In the hour previous to injection there was a fall of 10 mg. It is only possible, not probable, that in this animal there was a deposition of liver glycogen. Because of the difficulties of conducting this experiment and evaluating the results no further observations have been made.

Muscle glycogen deposition

Because of the integral part which muscle glycogen plays in carbohydrate metabolism its amount was determined in this series of hypophysectomized animals. Chart 9 shows the average values of muscle glycogen

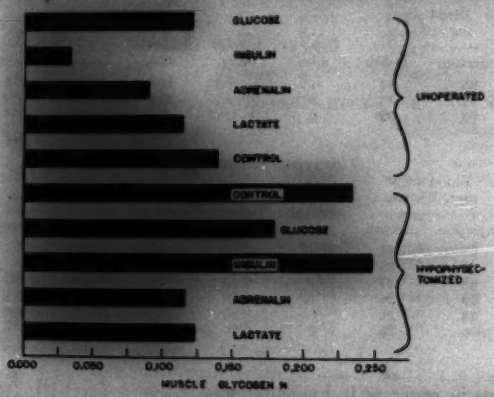

Chart 9. Average muscle-glycogen values of unoperated and hypophysectomized baby rabbits.

for the unoperated and the hypophysectomized controls and the animals given glucose, lactate, adrenaline and insulin. The special precautions necessary for careful muscle glycogen determinations were not taken. The animals were decapitated, so that a certain amount of muscle clonus occurred with reduction in muscle glycogen. Almost invariably the second sample of muscle glycogen was lower than the first, an interval of ½–1 min.

intervening between the taking of the two samples. The determinations are, therefore, inaccurate as to the amount actually present, but they do show that in the hypophysectomized baby rabbit's muscle glycogen is present in approximately normal amounts. Too much significance is not to be placed upon the minor differences. The failure of the hypophysectomized animal to maintain his liver-glycogen and blood-sugar level is not due to the absence of muscle glycogen.

Control operations

As has been stated, the operative approach to the pituitary through the base of the sella turcica, if performed with sufficient care, can eliminate brain trauma completely. There is evidence that operative manipulations of brain tissue in the region of the pituitary are in themselves accompanied by permanent brain damage and changes in the carbohydrate balance [Chaikoff *et al.* 1935]. Operative controls were made in order to exclude the various operative procedures and subsequent stomach tube feedings as a source of the changes found. All manipulations were made to expose the pituitary, but the gland was not removed. Table VI

Table VI. Operative controls

	No.	Blood sugar mg./100 c.c.		Interval min.	Glycogen p.c. Liver	Glycogen p.c. Muscle
Untreated	65	140		—	1·500	0·075
	70	33		—	0·000	0·000
		Injection	Death			
Adrenaline subcutaneous	69	87	224	41	1·980	0·329
	71	143	174	44	0·371	0·122
		Descent	Death			
Insulin intravenous	72	111–64	97	79	7·070	0·587
	74	121–62	69	90	6·370	0·122

shows that five out of six animals, treated and untreated, had normal amounts of blood sugar, liver and muscle glycogen. The exception, No. 70, behaved like the hypophysectomized animal. The fasting blood-sugar level was in the convulsive range, and no liver or muscle glycogen was found. At autopsy the entire pituitary was found compressed and necrotic, completely surrounded by wax. The injection of hot wax into the sphenoid bone is one of the operative procedures. Occasionally the wax escapes into the sella turcica with consequent destruction of the pituitary. This had occurred in this animal and the animal was essentially

hypophysectomized. It had not occurred in any of the other five operative controls. The operative and experimental procedures are, therefore, not a cause of the changes found in the hypophysectomized animals.

Conclusions

The diminished endogenous production of carbohydrate in the absence of the pituitary, first found by Houssay, is consistently and readily observed in the baby rabbit.

The importance of the liver to the control of the blood-sugar level in the hypophysectomized animal is demonstrated.

In the fasting state the blood-sugar level is maintained until the liver glycogen, stored from exogenous sources, becomes depleted. This depletion in the fasting baby rabbit occurs in a very few hours. In the absence of liver glycogen there is a rapid fall in blood sugar to convulsive levels. The similarity of this rapid fall in the hypophysectomized rabbit to that seen in the hepatectomized rabbit is striking.

Glucose utilization is not impaired, and liver glycogen is readily stored from glucose given intravenously. Adrenaline and insulin do not cause storage of liver glycogen as they do in the normal animal. The utilization of intravenous lactate is probably impaired. The failure of the hypophysectomized baby rabbit to maintain his liver-glycogen and blood-sugar level is not due to a diminished quantity of muscle glycogen.

The work reported in this paper is the direct outgrowth of studies done at the National Institute for Medical Research under the guidance and inspiration of Sir Henry Dale.

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ADRENALINE AND THE BLOOD LACTIC ACID LEVEL IN HYPOPHYSECTOMIZED RABBITS

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(*Received* 10 *August* 1936)

In the previous paper [Cope, 1936] it has been shown that adrenaline in quantities that store glycogen in the liver of the normal baby rabbit fails to do so in the hypophysectomized rabbit. Since muscle glycogen was present in normal amounts in the hypophysectomized animal, this suggested that the failure of adrenaline to store liver glycogen was either that it failed to break down muscle glycogen into lactic acid or that the lactic acid so elaborated was not utilized as in the normal animal. In order to determine at which point the action of adrenaline failed, it was decided to study the rise in the blood lactate in rabbits before and after hypophysectomy.

Marenzi [1934] working with Houssay has shown that the basal blood level of lactic acid is normal in the hypophysectomized dog, and that following injection of alkaline anterior pituitary extract the basal level rises [Houssay, 1936]. Chaikoff *et al.* [1935] have shown that the blood lactic acid rises following adrenaline in the hypophysectomized dog but does so to a lesser extent than in the normal.

Methods

West's modification [1931] of the Friedemann, Cotonio and Shaffer method has been used for the lactate determinations. 5 c.c. of blood are necessary for duplicate determinations and 3 c.c. if only one determination is made. In order to reduce the factor of blood loss to a minimum, the majority of the curves were made by removing 5 c.c. on alternate samples only, contenting ourselves with a single determination for the second, fourth and sixth samples. For the same reason, adult rabbits weighing 2–3·5 kg., not baby rabbits, were used in these experiments.

Arterial blood only was used, taken from the ear artery with a No. 27 needle and oiled syringe. In initial experiments cardiac punctures were used, but the great variations in almost simultaneous punctures suggested that at times the needle removed the blood from the left side, at times from the right side, of the heart. In order to exclude differences in venous and arterial blood and utilization of lactate by the lung [Evans *et al.* 1933], only those experiments in which peripheral arterial blood was used are quoted.

Blood-sugar determinations by the micro method of Folin [1932] were made on the same sample of arterial blood. 0·2 mg. of adrenaline (Parke, Davis and Co., 1:1000 solution of hydrochloride) in 1 c.c. of physiological saline was given subcutaneously following the removal of the control blood sample. The area of injection was gently massaged for 10 sec. after injection.

Hypophysectomy was carried out by a modification of the buccal approach to be described in detail elsewhere. The two principal lobes, stalk, and the pars tuberalis were removed in entirety effecting, therefore, a total hypophysectomy. No attempt was made to distinguish between anterior and posterior pituitary function, since it is impossible in the rabbit to remove one lobe and leave the other without disturbing its anatomical integrity. Since considerable experience has been attained with this method completeness of the removal of pituitary tissue can be assured at the time of operation by observation through the dissecting microscope. In the adult animal because of the development of the diaphragma sellæ it is difficult to remove the base of the stalk and the pars tuberalis, but with care this can be accomplished. The four animals which survived the final experiments were observed while going into spontaneous hypoglycæmic convulsions. For the purposes of these experiments this is as good a proof of the completeness of hypophysectomy as serial sections of the entire pituitary region.

From 1 to 6 weeks were allowed between hypophysectomy and post-operative observations.

Experiments

Blood lactic acid response to adrenaline. The arterial blood sugar and lactic acid were determined for a 3-hour period following the injection of 0·2 mg. of adrenaline subcutaneously. The control sample was taken and then samples were withdrawn ½, 1, 1½, 2 and 3 hours following the injection of adrenaline. In all the later experiments the 1½-hour sample was omitted in order to reduce the volume of blood withdrawn.

Every effort was made to eliminate in so far as possible all conditions which might alter the blood-lactate value. Before the observations each animal was handled as gently as possible. The animals were kept in blood-taking boxes during the entire experiment, and whenever an animal showed any excitement upon being placed in the box, the experiment was either postponed for an hour or two or until a subsequent day. Initial blood-sugar and blood-lactate values varied so markedly in the non-fasted control animal that all recorded control observations were made after an 18-hour fast. As has been previously observed [Cope & Marks, 1934], it was impossible to fast the hypophysectomized animals. In spite of this precaution, one animal, No. 5, had a blood-sugar value at convulsive levels at the onset of the experiment.

TABLE I

Rabbit No.	Blood lactate (mg./100 c.c.) Hours after adrenaline subcutaneous						Blood sugar (mg./100 c.c.) Hours after adrenaline subcutaneous					
	0	½	1	1½	2	3	0	½	1	1½	2	3
Before hypophysectomy												
2	32	32	38	42	40	70	103	142	169	194	215	250
5	22	28	41	74	38	32	107	139	200	210	217	235
13	6	35	17	—	19	18	80	103	135	—	165	179
56	5	25	48	—	32	22	89	154	182	—	202	180
	4	15	23	—	46	26	92	117	144	—	172	180
58	1	9	23	—	—	8	88	106	114	—	—	91
59	13	48	20	—	21	15	88	139	175	—	200	220
60	11	21	46	—	48	32	90	129	144	—	151	145
After hypophysectomy												
2	43	110	133	—	—	—	92	99	108	—	—	—
5	10	10*	106	—	—	—	38	33*	48	—	—	—
13	22	38	46	—	—	—	88	100	105	—	—	—
56	20	13	13	—	8	16	92	101	112	—	118	118
58	7	14	244	—	21	23	106	144	185	—	241	253
59	10	9	39	—	8	9	79	132	129	—	120	109
60	1	9	6	—	18	21	99	108	134	—	151	146

* Spontaneous hypoglycæmic convulsion occurred 7 min. before these readings, 23 min. after adrenaline injection.

Twenty-nine control observations were made on twenty-four different animals (Tables I, II, III). Considerable variation was found in the blood-lactate response. In twenty of the observations a rapid rise and subsequent fall occurred within the 3 hours, consistent with the observations in the literature. The time, however, at which the peak occurred varied in these twenty. In three the peak was reached in ½ hour, in ten in 1 hour, in three in 1½ hours, while in four it was not reached until 2 hours after injection. In the remaining nine observations definite

TABLE II. Controls. Twenty control observations in the 16 rabbits not observed after hypophysectomy

	Blood lactate (mg./100 c.c.) Hours after adrenaline subcutaneous					Blood sugar (mg./100 c.c.) Hours after adrenaline subcutaneous					
No.	0	½	1	1½	2	3	0	½	1	1½	2	3
1	44	64	—	96	58	2	69	89	—	101	115	108
3	16	42	42	43	50	50	88	142	171	183	210	202
4	26	107	216	152	104	24	111	142	187	208	222	217
6	66	98	98	210	107	66	109	160	190	215	—	235
7	194	—	228	—	38	—	107	167	180	—	186	164
	87	—	287	268	186	145	91	—	132	141	165	161
8	93	80	52	—	33	23	97	155	222	—	149	—
	132	200	225	—	198	83	116	217	247	—	282	282
9	198	66	156	—	84	34	100	120	127	134	142	154
	113	99	73	—	31	26	127	154	164	—	213	222
	59	52	64	—	74	—	124	136	168	—	222	—
10	32	39	—	62	71	93	84	103	150	163	174	188
11	10	40	27	—	20	9	101	148	179	—	206	210
12	7	12	12	—	—	13	113	130	144	—	183	215
14	56	139	75	—	25	20	123	148	172	—	188	196
15	90	127	131	—	71	93	106	140	152	—	191	215
55	38	12	8	—	9	49	95	110	111	—	127	139
57	33	35	57	—	—	28	110	157	179	—	—	220
62	11	17	58	—	42	21	104	145	180	—	217	220
63	26	—	34	—	37	17	92	118	147	—	159	180

variations from the classical response were encountered. In three there was a continuous, moderately sharp rise throughout the 3 hours. In two there was a very slow continuous rise. In three a continuous fall occurred. In one there was an initial fall followed by a rise in the third hour. In the three animals in each of which two control observations were made, in only one, rabbit No. 56 (Table I), were the two curves of the same general character. In the one animal in which three control observations were made no two were alike (Table II).

The response of the blood lactate to adrenaline did not depend upon the initial blood lactic acid level. In seven observations in four animals the initial level was 87 mg. or more. In four of these observations the classical response occurred.

No constant relation of the blood lactic acid to the rise in blood sugar was determined. The blood sugar rose consistently in all animals, a minority reaching a peak at the end of the second hour, the majority still rising in the third hour. Because of the constancy of the blood-sugar curve it is probable that the variations in the blood-lactate curve did not depend upon a difference in the rate of absorption of the adrenaline.

Only seven of the rabbits were in sufficiently good condition for observations after hypophysectomy (Table I). The first three died during these observations from blood loss before the end of the third hour. In all three a significant rise in blood lactate as well as blood sugar had occurred by the end of the second hour. In animal No. 5, whose blood sugar was low at the onset of the experiment, a convulsion occurred in spite of the adrenaline. Seven minutes after the convulsion the blood lactic acid was still

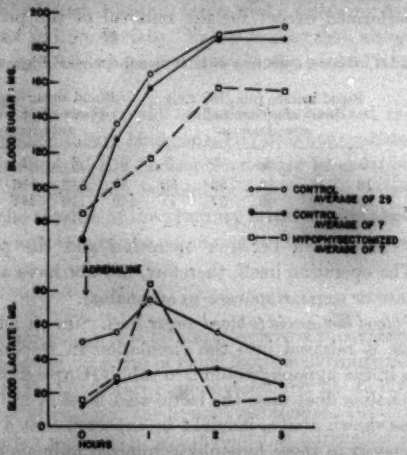

Chart 1. Average blood-sugar and blood lactic acid curves following injection of 0·2 mg. adrenaline. Open circles represent average of all twenty-nine control observations. Closed circles represent average of eight preoperative observations in the seven animals observed after hypophysectomy. Open squares represent the seven observations after hypophysectomy.

at the initial level, suggesting that no large amount of lactate was released into the circulation by the convulsion. The later high rise in blood lactic acid of course may have been in part due to a delayed release of lactate formed by the convulsion as well as to the direct effect of adrenaline upon the muscle glycogen. In the four animals which survived in good condition the withdrawal of blood, three showed a very definite rise in blood lactate, and the fourth, No. 56, showed an initial fall followed by a rise in the third hour. The variations following hypophysectomy were no greater than those found in the controls. That there

is a rise in blood lactate following adrenaline in the absence of the pituitary there can be no question. This rise in the rabbit is of the same magnitude as before hypophysectomy.

The blood-sugar curves following hypophysectomy were altered as previously reported by Corkill *et al.* [1933], although in no animal did a rise fail to occur (Chart 1). In general there was a diminished response.

The observations before and after a control operation show no significant variations (Table III). In this animal the entire operative procedure was performed except for the removal of the pituitary gland.

TABLE III. Control operation without actual removal of hypophysis

	Blood lactate (mg./100 c.c.) Hours after adrenaline subcutaneous					Blood sugar (mg./100 c.c.) Hours after adrenaline subcutaneous				
Rabbit No. 61	0	½	1	2	3	0	½	1	2	3
Before operation	18	17	36	63	56	99	123	156	230	204
After operation	10	13	66	37	17	110	116	142	164	171

Eight days were allowed between operation and the post-operative observation. The operation itself, therefore, did not have an effect upon the blood lactate or sugar responses to adrenaline.

Relation of blood lactic acid to blood-sugar level. Since it has been shown that adrenaline is released into the circulation in response to insulin hypoglycæmia in the hypophysectomized rabbit [Cope & Marks, 1934], and since adrenaline does raise the lactic acid level of the blood in the same animal as shown above, it might be expected that a rise in blood lactate would occur in those hypophysectomized animals allowed to go into spontaneous hypoglycæmia.

In the control observations the initial or fasting blood level of lactic acid varied very considerably from 198 to 1 mg., with an average of 60 mg. (Tables I, II, III). The fasting blood-sugar level showed no significant difference in those animals with a high fasting level of blood lactic acid. The average non-fasting blood lactic acid level for the hypophysectomized animals before adrenaline injection was 16 mg., varying from 43 to 1 mg. The values showed no consistent variation with the blood sugar. In rabbit No. 5 the initial blood-sugar level was 38 mg., definitely a preconvulsive level. The blood lactic acid was only 9 mg., well below the average for all the hypophysectomized animals (Table I).

In the previous paper [Cope, 1936] the blood lactic acid value was given in ten hypophysectomized baby rabbits. In six observations in the uninjected animals the lactic acid varied from 13 to 95 mg. (Table I).

Although the average level of the blood lactic acid in the three animals with a blood sugar of 60 mg. or below was 47 mg. and in those above 60 mg. was 28 mg., too great a variation occurred in this small series to make the averages significant. In two animals with blood sugars above 260 mg. following glucose administration the blood lactic acid values were 41 and 68 mg., well within the limits of normal controls. A convulsion had not occurred in these two animals previous to the glucose administration. Blood lactic acid values of 247 and 254 mg. were obtained in two more animals, 81 and 40 min. respectively following intravenous administration of sodium lactate. Values higher than either of these two were encountered following adrenaline in the control adult animals of this paper.

Since the blood lactic acid in hypophysectomized rabbits showing spontaneous preconvulsive blood-sugar levels is not consistently increased over those with normal non-fasting blood-sugar levels, it is probable that any amount of adrenaline released in response to spontaneous hypoglycæmia in the absence of the pituitary is less than is absorbed from the subcutaneous injection of the 0·2 mg. given in these experiments.

Summary and conclusions

In rabbits with the whole pituitary removed subcutaneous adrenaline injection is followed by a rise in blood lactic acid comparable in duration and amount to the rise before hypophysectomy. The failure of adrenaline to deposit liver glycogen in the hypophysectomized baby rabbit is, therefore, presumably not due to the inability of adrenaline to break down muscle glycogen to lactic acid.

Since the normal rise in blood sugar as well as the deposition of liver glycogen following adrenaline do not occur in the absence of the pituitary, the normal rise in blood lactic acid in the hypophysectomized rabbit is further evidence that the pituitary plays a role in lactate utilization.

The blood lactic acid is not significantly increased in hypophysectomized rabbits fasted until their blood sugar is below 40 mg. If, therefore, adrenaline is released into the circulation in response to spontaneous hypoglycæmia in the absence of the pituitary, it is in smaller amounts than result from the absorption of 0·2 mg. given subcutaneously.

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THE STUDY OF THE CAROTID SINUS RESPIRATORY REFLEXES BY MEANS OF CHRONIC EXPERIMENTS

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(*Received* 17 *August* 1936)

The number of experiments of a chronic nature which have been carried out on the carotid sinus respiratory reflexes has been comparatively small, although it has been generally recognized that these experiments have certain advantages over acute experiments, in that one can eliminate the effects of anæsthesia on the respiration, the interference with vital centres caused by procedures such as decerebration, and the general shock of a surgical operation. A chronic experiment is one in which the respiratory reflexes are studied only after the animal has completely recovered from the shock of the operation for denervating the vaso-sensory areas. Since the main purpose of these chronic experiments is to examine the animal under approximately physiological conditions, it is necessary to find means of studying respiration, which will cause a minimum amount of shock, fright or interference with normal functions.

Gemmill & Reeves [1933], in chronic experiments in dogs with masked breathing, found in accordance with the work of Schmidt [1932] in acute experiments, that the dog after denervation of the vaso-sensory areas no longer showed an increase in pulmonary ventilation in response to anoxia, but rather a decrease, while the hyperpnœa in response to increased CO_2 was unchanged. Gemmill, *et al.* [1934], continuing this work, found that in response to lesser degrees of anoxia over longer periods, some degree of stimulation in the chronically denervated animals was seen. Green & de Groat [1935], also using mask breathing in dogs, found that the response of the denervated dog to CO_2 was less than that of the normal animal. Wright [1936] carried out chronic experiments on the rabbit, and found the same depression of respiration in denervated animals in response to anoxia

which he had previously obtained in acute experiments [1934], but this was only seen if the respiratory tests were done under anæsthesia. He also found that these animals responded to increased CO_2 by hyperpnœa. The author [1936], also working on chronically denervated rabbits, found a marked depression of respiration in response to anoxia even in the unanæsthetized animal. In this case also it was seen that the rabbits responded to excess CO_2 by hyperpnœa. The present paper is a more complete account of these results, and of further work along similar lines.

Investigations have also been made on the respective roles of the carotid sinus and aortic areas in the respiratory reflexes of the rabbit. Schmidt [1932] and Wright [1934] both concluded from acute experiments that in the rabbit the aortic areas play no significant part. In the case of the dog, Cordier & Heymans [1935], reviewing previous work, attribute to the aortic areas an important role. I have therefore compared the responses of rabbits with varying degrees of denervation of the vaso-sensory areas.

A further point which it was desired to investigate was the combined effect of anoxia and CO_2 on the denervated animal. This has not previously been investigated in chronic experiments, while in acute experiments Selladurai & Wright [1932 *a*] found that anoxia depresses the response of the denervated animal to CO_2.

Experimental procedure

The general procedure used in this work was to denervate various parts of the vaso-sensory areas at a one-stage operation under ether anæsthesia, and after complete recovery of the rabbit from the operation to record the response to breathing various gas mixtures. The extent of the operations, with the number of animals indicated in brackets, is shown below.

Group I.
- (*a*) Both sinuses denervated and both depressor nerves divided. (4)
- (*b*) Both sinuses denervated, both depressor nerves, both cervical sympathetics, and right vagus divided. (3)

Group II.
- (*a*) Both depressor nerves divided. (3)
- (*b*) Both depressor nerves, both cervical sympathetics, and right vagus divided. (3)

Group III. Both sinuses denervated. (3)

There were thus three main groups of animals: (1) those where both sinuses and aortic areas were denervated; (2) those where the aortic

areas were denervated and the sinuses left intact; and (3) those where the aortic areas were left intact and the sinuses only denervated.

Denervation of the carotid sinuses was done by cleaning the carotid bifurcation and ligaturing the internal carotid artery just at its origin and also about ½ in. more distally. In operations involving cutting of nerves a section of about ½–1 in. was removed from the nerve.

The animal was always allowed at least 2 weeks to recover from the operation before any investigations on respiration were carried out. The investigations consisted in recording the response of the rabbit to various gas mixtures, namely (*a*) air containing varying amounts of CO_2, (*b*) mixtures of air and nitrogen, and (*c*) mixtures of air, nitrogen and CO_2. The administration of the gas mixtures and the recording of the respiration was done by the method I have previously

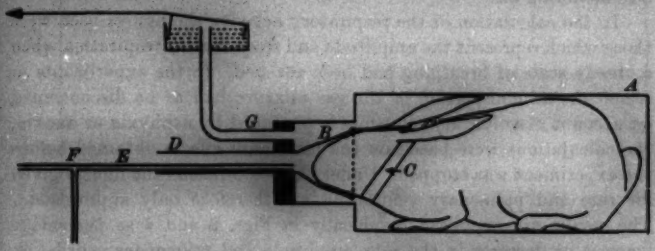

Fig. 1. Apparatus for recording respiration of the rabbit.

described. It is illustrated in Fig. 1. The rabbit was enclosed in an airtight chamber *A*. A rubber mask *B*, kept in position by a leather collar *C*, fitted closely over the rabbit's face. This mask communicated with a glass tube *D*, which led through the cover of the chamber. Inside this tube was a finer tube *E*, by means of which gas mixtures could be introduced into the mask. By means of the two-way stopcock at *F* the gas mixture being administered could be instantly replaced by another. A second tube *G*, leading through the cover of the chamber, communicated with a float volume recorder, which thus measured the volume change in the animal with each respiration. This was taken as the amplitude of respiration. It should be stressed again that the rabbits submitted quietly to this procedure without dyspnœa or struggling, and the even regular tracing of the respiration obtained suggested that the animal was under reasonably normal physiological conditions. By this method it was possible to measure the rate and

amplitude of respiration, and hence the pulmonary ventilation, without the use of an anæsthetic and without any operative interference such as tracheotomy.

Each experiment was commenced by recording the breathing while air was being circulated through the mask. After a few minutes the air was turned off and the gas mixture administered. The administration of the gas lasted about 1–2 min., except in the case of large percentages of CO_2 or extreme degrees of anoxia, in which cases the administration was discontinued when the animal showed signs of discomfort, or the respiration was depressed to a very severe extent. Apart from these cases the breathing appeared to reach a state of equilibrium in the short time the experiment lasted. The experiment could be concluded at any moment by turning off the gas mixture and administering air.

In the calculation of the respiratory activity the figures used were those which represent the amplitude and frequency of respiration when a steady state of breathing had been reached. In the experiments in which the administration of the gas mixture had to be discontinued on account of voluntary movements produced by asphyxia or anoxia, the calculations were based on the maximum effects obtained before the experiment was stopped. Under these conditions the figures given for rate and pulmonary ventilation are therefore only approximate. The results are expressed graphically in Figs. 3 and 6 as percentage increase or decrease in the rate, amplitude and pulmonary ventilation in response to the gas mixture given, in comparison with the values obtained during the administration of air.

Results

Group I. Animals with both sinuses and aortic areas denervated

As was indicated above this group includes two sets of animals. In one of these denervation of the aortic areas was incomplete since both cervical sympathetics and vagi were left intact. These animals, however, were the first to be studied, and the results obtained from them illustrate the most important findings. Owing to the criticism that there was incomplete denervation of the aortic areas, the second set of operations was done later, in which, in addition to denervation of the sinuses and division of the depressor nerves, the cervical sympathetics were cut and also the right vagus. The results obtained from these animals differed in no way from the first set, the responses to

Fig. 2. Record showing the response of the rabbit to 8 p.c. CO_2 after denervation of the carotid sinuses and aortic areas. The time tracing is marked in seconds.

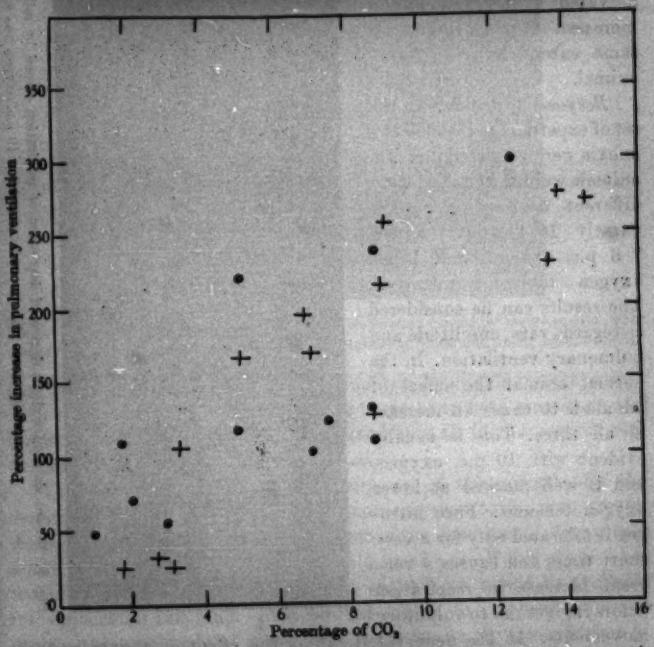

Fig. 3. Graph showing the percentage increase in pulmonary ventilation in response to increased amounts of CO_2 in the air breathed in normal and denervated rabbits. ⊕ represents normal animals. + represents denervated animals.

CO_2 excess and anoxia being similar to those in the first set. No separate records or figures for these animals are therefore given.

Response to CO_2. It was found that the denervated rabbit can still respond to increased CO_2 by increasing its respiration. Fig. 2 shows a record illustrating this. The quantitative study summarized in Fig. 3 shows that the denervated rabbit responds to increased CO_2 to much the same extent as the normal animal.

Response to anoxia. In this set of experiments the effects of anoxia were very striking. The animals were tested with three different degrees of anoxia, namely 10–11 p.c. oxygen, 7–8 p.c. oxygen, and 1 p.c. oxygen (cylinder nitrogen). The results can be considered as regards rate, amplitude and pulmonary ventilation. In the normal animal the effect of anoxia is to cause an increase in all three. This is usually evident with 10 p.c. oxygen, and is well marked at lower oxygen tensions. Pure nitrogen is tolerated only for a very short time, and causes a very great increase in respiration before it gives rise to voluntary movements. In the denervated rabbit the effect of anoxia is quite different. The frequency of respiration is only slightly increased even by extreme degrees of anoxia. The amplitude, on the other hand,

Fig. 4. Record showing a slight degree of depression of respiration caused by mild anoxia in the denervated rabbit. At the arrow a gas mixture containing 10 p.c. oxygen was administered.

shows a very definite depression. In severe states of anoxia, as I have previously reported, it may fall to 30 p.c. of its original value. This depression of the amplitude is usually evident with gas mixtures containing less than 10 p.c. of oxygen. The combined effect of these is to produce a depression in the pulmonary ventilation, since the depression of the amplitude outweighs the small increase in frequency. Records showing the effects of anoxia on the denervated rabbit are shown in Figs. 4 and 5. Fig. 6 shows the combined results of a number of experiments on the effects of different degrees of anoxia on normal and denervated animals. From this can be seen (*a*) the small increase in

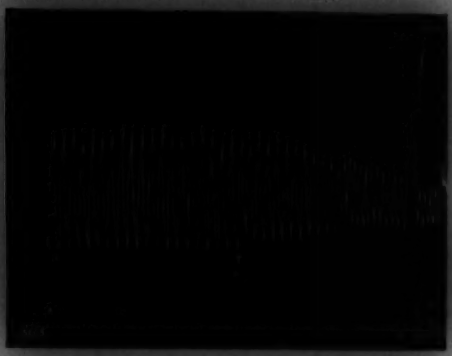

Fig. 5. Record showing the great depression of respiration caused by severe anoxia in the denervated rabbit. Cylinder nitrogen was administered containing about 1 p.c. oxygen.

rate in response to anoxia in the denervated animals in comparison with the normals, and (*b*) the definite decrease in both amplitude and pulmonary ventilation in the denervated animals in comparison with the large increase in the normal animal.

The difference between the respiration of the normal and the denervated animals in response to anoxia is seen to be even greater if one takes into consideration the effect of the dead space. An average figure for the tidal air in a rabbit would be 12 c.c. Of this about 3 c.c. would occupy the dead space, so that about 9 c.c. would be available for ventilating the lung. In a normal rabbit, in response to severe anoxia the amplitude of respiration might be nearly trebled, that is, it would amount to 36 c.c. As the dead space is believed to alter very little, about 33 c.c would be available for ventilating the lung. This is

almost four times the normal ventilation, although the amplitude has only been increased threefold. On the other hand, in a denervated rabbit under similar conditions, the amplitude of respiration might fall to one-third of its original size, that is, to 4 c.c. Of this 3 c.c. would still be required to fill the dead space, so that only 1 c.c. would be available for ventilating the lung. The depression of alveolar respiration is in this case much greater than would at first appear.

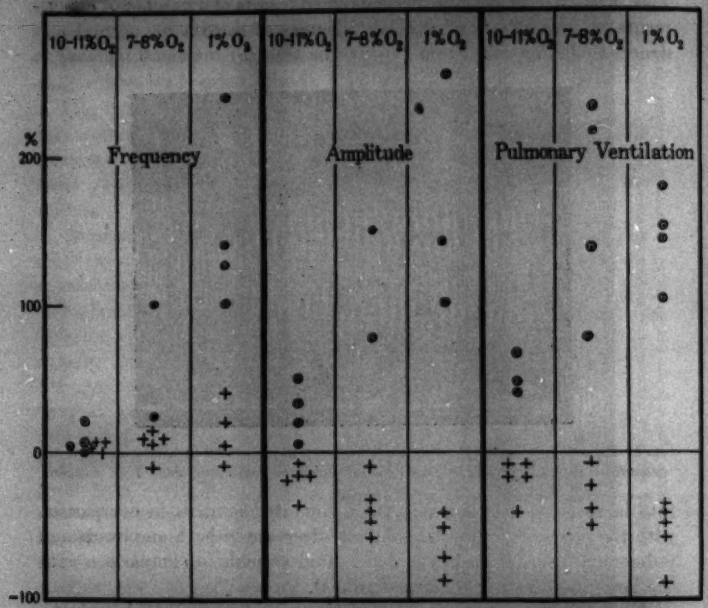

Fig. 6. Table showing the effect of various degrees of anoxia on the amplitude and frequency of respiration and on the pulmonary ventilation in normal and denervated rabbits. ⊙ represents normal animals. + represents denervated animals.

Group II. Animals with only aortic areas denervated

This group also includes two sets of animals: (*a*) those where only the aortic nerves were divided, and (*b*) those where a more complete denervation of the aortic areas was obtained by dividing also the sympathetics and the right vagus. It was found that both sets of

animals responded in the same way, and the more complete denervation did not cause any different effect from that produced by simply dividing the depressor nerves.

Since no difference in the response to CO_2 was found in the animals in group I and in normal animals, it is unnecessary to consider the effect of CO_2 on the animals in group II. More important is the response to anoxia. Experiments on all six rabbits showed that these animals respond to anoxia by increasing the amplitude and frequency of respiration. In no case was evidence of depression obtained. Since the response is the same as that of normal rabbits, it would seem that the aortic areas do not play any important part in the response of the rabbit to anoxia.

Group III. Animals with only sinuses denervated

The rabbits with only the carotid sinuses denervated and the aortic areas left intact were found to behave in response to CO_2 and anoxia in a similar way to the animals in group I, where both sinuses and aortic areas had been denervated. These rabbits respond to excess CO_2 by increasing the amplitude and frequency of their respiration, but in response to anoxia they show a depression instead of a stimulation of breathing. This is consistent with the results obtained in the animals in group II, and indicates that the sinuses alone are responsible for increased respiration caused by anoxia in the intact animal, and that the role of the aortic areas in this reflex is negligible.

Combined effects of anoxia and CO_2 excess

A few experiments were carried out to compare the effect of CO_2 excess combined with anoxia on normal animals and the denervated animals in group I (*a*). The effect of CO_2 excess combined with anoxia is greater in the normal rabbit than that of the same percentage of CO_2 without accompanying anoxia. Mixtures of 7 p.c. oxygen and 5 p.c. CO_2, and also 1 p.c. oxygen and 5 p.c. CO_2 both stimulated the respiration vigorously as regards amplitude and frequency. In the case of the denervated animals the stimulation of breathing by these mixtures was much smaller, while in one case 1 p.c. oxygen and 5 p.c. CO_2 caused a definite depression in amplitude with no change in the frequency. The effect of these mixtures on the denervated rabbits was always less than that of the same percentage of CO_2 in air. It appears therefore that, in the normal rabbit, anoxia increases the response to CO_2, while in the denervated rabbit anoxia reduces the response to CO_2.

Discussion of the results

The results obtained show that the denervated rabbit can respond to increased amounts of CO_2 in the same way as the normal, i.e. by increasing its respiration. This indicates that CO_2 can act centrally in the absence of the carotid sinus reflex pathway. This is supported by the chronic experiments of Gemmill & Reeves on dogs, and of Wright on rabbits; and also by the acute experiments of Schmidt. On the other hand, Selladurai & Wright [1932 *b*] found a diminished response to CO_2 in denervated cats. Cordier & Heymans [1935] have concluded that CO_2 can act either reflexly or by a direct action. The question of interest is which of these mechanisms is actually used in the animal, and here the weight of evidence from chronic experiments is in favour of a direct action on the centre. Such evidence must be regarded as of considerable value, since the conditions of working are much nearer physiological conditions than is the case in acute experiments.

As regards the action of anoxia, the results found here support those of most of the other experiments both acute and chronic, in that the hyperpnœa in response to anoxia is seen to depend on the integrity of the carotid sinuses. Certain results from chronic experiments fail to agree with this conclusion. Dechaſneux [1934] found that dogs after denervation of the vaso-sensory areas, on being taken to high altitudes, showed an increase in breathing. Gemmill, *et al.* [1934] found a hyperpnœa in denervated dogs in response to small degrees of anoxia over long periods. Cordier [Cordier & Heymans, 1935] reports that denervated dogs exhibit increase in respiration in response to gradually progressive anoxia. All these results refer to small degrees of anoxia of gradual onset. This type of anoxia I have not investigated. The points to be emphasized in my results are the very great depression of respiration caused by the direct action of anoxia on the respiratory centre of the rabbit, and also that this effect has been obtained in unanæsthetized animals, in which circumstances Wright [1936] had failed to obtain any evidence of depression. From all these results it seems probable that acute anoxia stimulates breathing through the carotid sinus reflex, while anoxia of gradual onset may directly stimulate the respiratory centre.

From the experiments on the combined effect of CO_2 and anoxia it has been seen that, in the denervated animal, the response to CO_2 is reduced in the presence of anoxia. This suggests that anoxia acting

directly on the respiratory centre reduces the sensitivity to CO_2. This supports the results of Selladurai & Wright [1932 *a*] who pointed out that their findings were opposed to the old view of Herman that oxygen lack sensitizes the centre to the action of CO_2.

Finally the results support the view of Schmidt and also of Wright that in the rabbit the aortic areas play no significant part in the respiratory reflexes.

Summary

1. An investigation of the effects of chronic denervation of the vaso-sensory areas on respiration in the rabbit is described.

2. Rabbits, after denervation of the carotid sinuses and aortic areas, respond to excess CO_2 by increasing their respiration to approximately the same extent as the normal animals.

3. Anoxia produces in the denervated rabbit a depression of respiration. The frequency may be slightly increased, but much less than in the normal animal, while the amplitude is greatly diminished. The total effect is a diminution of the pulmonary ventilation.

4. Anoxia reduces the response of the "isolated" respiratory centre of the rabbit to CO_2 excess.

5. In the rabbit the aortic areas play no significant part in the respiratory reflexes. The carotid sinus regions are the principal chemoreceptors to anoxia.

I am indebted to Prof. H. Barcroft for his advice and assistance during this work.

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THE ELECTRICAL RESPONSES OF DARK-ADAPTED FROGS' EYES TO MONOCHROMATIC STIMULI

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(*Received* 19 *August* 1936)

With the aid of the slow d'Arsonval galvanometer and the dispersion spectrum of a gas lamp, Himstedt & Nagel [1901] showed that a phenomenon, corresponding to the well-known Purkinje shift, could be obtained with the "mixed" eye of the frog. In dark adaptation and with weak stimuli maximal electrical responses were elicited at 0·544 μ; after adaptation to bright sunlight the maximum shifted to 0·590 μ and strong stimuli had to be used. The results were confirmed and extended to other eyes by Piper [1904, 1905] who used a similar instrument and the spectrum of a Nernst lamp. In the work of Brossa & Kohlrausch [1913], with the faster string galvanometer, the various phases of the electroretinogram were separated and the old results shown to hold good for the *b*-wave of the electrical response. The literature up to 1931 has been summarized by Kohlrausch [1931].

The recent results of Charpentier [1936] have served to simplify the problem. With the rod eye of the albino rat he found that the electrical responses disappear after complete bleaching of the visual purple in sunlight. During V.P. regeneration in darkness the *b*-wave returned and increased in size along the curve determined by Tansley [1931] for regeneration of visual purple in the rat, the height of the *b*-wave corresponding to the concentration of V.P. Now, in the "mixed" eye of the frog, electroretinograms may still be obtained after adaptation to bright sunlight. Thus the old work, referred to above, means that rods and cones separately give rise to *b*-waves so that in work with monochromatic stimuli little can be gained by using, for example, a high intensity and a dark-adapted "mixed" eye. In such cases the rod *b*-waves add themselves to the cone *b*-waves, and the broad band of maximal responses in the middle of the visible spectrum, which is thereby obtained, is uninterpretable in terms of photochemical events in the two types of receptors.

In the old work with the frog's eye the state of adaptation relative to intensity of stimulation was controlled, but the important condition of an equal energy spectrum was neglected. In more recent work, where the latter factor has been observed, either the two states of adaptation have not been kept rigidly separated [Chaffee & Hampson, 1924; Smit, 1934], or the *b*-waves have not been systematically measured [Smit, 1934]. With six spectral regions, obtained with filters and equalized with respect to energy, Graham & Riggs [1935] and Graham, Kemp and Riggs [1935] have studied the initial phase of the electro-retinogram of the white rat and the pigeon.

Our original intention, in starting this work, was to collect and average for the two states of adaptation a great number of measurements of size and rate of rise of the *b*-wave, relative to an equal energy spectrum divided into narrow bands 0·010 μ apart. This plan has been carried out, but since in our routine experiments, new facts turned up after some 1500 potentials had been recorded with the dark-adapted eye alone, we decided to publish separately the results with light- and with dark-adapted eyes.

Technique

Apparatus and preparation

A straight filament quartz lamp, calibrated by the National Physical Laboratory for a mean colour temperature of 2800° K. was used as a light source for a Tutton monochromator. In this type of lamp (Phillips' tungsten) the filament is a single narrow band easily focused so as to fill the collimator slit. The lamp was calibrated in amperes, but adjusted, in its final position, for the corresponding voltage with the aid of an ammeter, kindly lent to us by the Physical Laboratory of this University, as in the range needed only a voltmeter was available in our own laboratory. Storage batteries were used to supply current to the lamp. The voltage was controlled between every exposure of the eye.

With the aid of Hg, Cd, and Cu spectra the dispersion curve of the monochromator was determined, and the exit slit finally adjusted so as to let through respectively 0·001 μ at 0·580 μ, 0·0006 μ at 0·500 μ, and 0·0013 μ at 0·630 μ. These adjustments were carried out through the eyepiece with the slit focused for 0·580 μ. The size of the exit slit and the focus were thereafter kept constant throughout the experiments, but the eyepiece was removed and replaced by two apochromatic lenses.

Together with the Tutton monochromator and extra apochromatic lenses, a wedge and an auxiliary non-adjustable density were obtained

from the makers (Adam Hilger, Ltd., London), both specially calibrated by them for selective absorption in the visible part of the spectrum. By setting the wedge (nonius readings) and, if necessary, inserting the auxiliary density, the intensity at any wave-length could be reduced by a known amount.

In order to obtain an equal energy spectrum the following procedure was adopted: using the tables computed by de Groot [1931] for shortening calculations with the well-known formula of Planck, the black body energy distribution at 2800° was obtained between 0·450 and 0·650 μ. The factor for converting black body radiation to tungsten, a minor correction, was located in a graph given by Hulburt [1917]. A further correction was obtained from the dispersion curve, determined in the manner described above. The final relative energies could now be equalized with the minimum amount of energy in 0·450 μ by adjusting the wedge which automatically brought in a correction for selective absorption in the standard wave-lengths used. The wedge settings were calculated in advance, and different levels of intensity obtained by putting in the auxiliary density or diminishing the collimator slit, for which certain standard apertures were used.

The bright lamp and the Tutton monochromator with its large prism and aperture ratio of *F*/6 combined to give strong stimuli, despite the narrow exit slit used, so that with dark-adapted eyes the collimator and exit slits were set at much the same values. The image of the exit slit, after removal of the ocular, was focused so as to fall on the retina of an excised frog's eye with its normal hypermetropia of about +6 D. Exposures of 1 sec., given by a Compur shutter, were used at higher intensities. At lower intensities 3 sec. were timed with a stopwatch. The longer duration at these intensities was chosen in order to ensure full development of the *b*-wave.

Values from the uncorrected region between 0·450 and 0·400 μ have been included in our results. In this region the stimuli are relatively too weak, but it is doubtful whether towards the violet end this factor actually is a very important source of error.

In our laboratory winter frogs are kept at a low temperature in the basement. The preparations were found to be more satisfactory, if the animals had been living for some time in a basin at room temperature. The results to be reported were obtained with winter and spring specimens of *R. esculenta* and winter specimens of *R. temporaria* which had been kept for some days at room temperature and dark adapted for not less than 12 hours. No differences were observed between the two kinds of frog.

The eyes were exised in red light and left for some 15–20 min. in the dark box with the electrodes inserted and oxygen bubbling through a capillary alongside the bulb. Preliminary experiments had shown that the dark-adapted eyes gave bigger and more regular responses in an atmosphere of oxygen.

Procedure

More than one level of intensity was rarely used with a given preparation, but at this level it was our aim to collect as many readings as possible at intervals in the spectrum of 0·010μ. At higher intensities exposures were taken every 3 min., at lower intensities every 2 min. Generally the order of the observations was determined in advance, so as to give the various wave-lengths and big and small responses an impartial distribution. This was particularly important in view of the fact that in most eyes the sensitivity first rose, then became steady, and finally began to fall. The interval of 20 min. in oxygen before the experiment began was calculated to cover the period of rapidly increasing responses, due partly to a slow dilatation of the pupil after the operation, but chiefly to after-effects of the operation itself and to the gradual oxygenation of the tissues.

At least at higher intensities the first stimuli seem to enhance the performance of the eye, a "staircase" effect noted previously by Brossa & Kohlrausch [1913] and by Smit [1934]. The pupil contracts during the operation independently of whether the eye has been stimulated with light or not. As is to be expected, atropine is of little use with the frog's eye, though in some experiments it has been tried. The pupil normally dilates in the dark box and is too slow to be influenced by short flashes of light some minutes apart.

The slow changes in the sensitivity as well as other factors, to be mentioned below, make it necessary to use one or two standard wave-lengths as controls and calibrate intervening observations in terms of the standards. Generally 0·510μ was used as control and given the value of 100. A systematic change in the control was distributed in equal steps over the interval, usually four or five observations. With observations as near as 0·010μ apart following in haphazard order we often have had opportunities of testing the correctness of the assumption made in this method of correcting the readings.

Most eyes, when they cease to respond normally, do so fairly rapidly. It is difficult to give any definite figures for the time a preparation may last, but it should last for at least an hour to be at all useful. We find that

we have averaged thirty observations with each preparation, but then several experiments have been discontinued merely because we have been limited by the time at our disposal.

I. General observations

Before describing the results of the measurements we want briefly to review a number of observations which, in many ways, have served to remodel our own conceptions both of the retinal responses and of the task undertaken in this work.

The multiple b-wave

A problem to which our results provide a definite answer was, we believe, first raised by Gotch [1903] and Einthoven and Jolly [1908], and concerns the origin of certain humps or extra waves sometimes to be seen on the retinal response to continuous stimuli. An important contribution to this question was made by Chaffee, Bovie & Hampson [1923] who observed that the initial *b*-wave of the frog's electroretinogram sometimes rises in two or more steps and that this phenomenon is more marked at lower intensities. Meservey & Chaffee [1927] with other types of eyes, Smit [1934], Granit & Riddell [1934] and others with the frog's eye, have described similar phenomena. But, when later Granit & Therman [1935] found that synchronized impulses in the optic nerve produce extra waves as artefacts on the retinal response, the existence of a multiple *b*-wave could not be held to be proved.

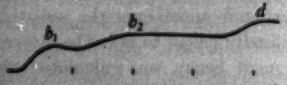

Fig. 1. Typical diphasic low intensity response showing b_1, b_2 and d (off-effect). Wave-length of stimulus 0·470 μ. Time in sec.

With weak stimuli, at or below the cone threshold (see section on measurements) we soon confirmed the fact that the *b*-wave often becomes polyphasic, usually diphasic. A typical diphasic low-intensity response is shown in Fig. 1. The *b*-wave rises in two phases, b_1 and b_2, and the response often ends in an off-effect (d). It is hardly ever possible to measure more than two phases though, at times, three steps may be seen.

It is easily shown that the slow b_2 actually is a *b*-wave and not identical with the well known *c*-wave, often termed "the slow secondary rise", which on the analysis of the retinal action potential [Granit, 1933] can be isolated as a specific component P I. At low intensities no typical *c*-waves can be seen, only b_1 and b_2, and sometimes the off-effect. But, as

the intensity is increased, a very large slow secondary rise appears after a latency of 6–8 sec. Now the latent period of b_2 is about equal to but not more than a second, b_2 reaches its top value in 2–3 sec., long before the *c*-wave becomes visible, and it is best seen as a separate entity at intensities below the threshold of the *c*-wave. Further, it is often possible, by carefully increasing the strength of the stimulus, to see b_1, b_2 and the *c*-wave simultaneously, though, as a rule, at higher intensities b_1 and b_2 unite in forming a single homogeneous *b*-wave. Generally also the *c*-wave requires a longer exposure than the two phases of the *b*-wave.

It is probable that the retinal response is sometimes modified by leakage from the nerve, but it is possible to show that in general b_2 is part of the retinal reaction to light. The *b*-wave is associated with the discharge through the optic nerve [Granit & Therman, 1935] so that if b_2 were an artefact from the nerve, then the interval between b_1 and b_2 would correspond to intraretinal delay. The latter, however, is of the order of magnitude of a few milliseconds [Granit & Therman, 1935], whereas the interval between the maxima of b_1 and b_2 may be a matter of 1–3 sec. On repetition of the same stimulus after an interval of some 15–30 sec., b_2 is often found to be much smaller than during the first exposure, whilst b_1 is practically unchanged. This experiment, when it succeeds, indicates different rates of recovery of b_1 and b_2. Direct evidence for the existence of several *b*-waves differing in latent period, size, and rate of rise and decay is provided by the phenomenon which we have termed "the switchboard effect".

The switchboard effect

By starting with the false assumption that all irregularities indicated a bad preparation, we very nearly missed the chance of finding this interesting group of phenomena. But as some eyes gave non-repeatable readings without in the least being fatigued or otherwise in a bad condition, we finally decided in some cases of this type to use only a few wavelengths instead of samples from the whole spectrum. It then became evident that in some eyes different types of responses may be elicited by the same stimulus.

The switchboard effect is most easily seen at low intensities (at or below cone threshold, as shown in section on measurements). The maximal *b*-waves around 0·500μ are then about 0·100 mV. In such cases the regularly repeated control at 0·510μ often shows a number of stages between (i) b_1 and b_2 separated in two clearly defined phases, (ii) the two *b*-waves combining themselves and giving together a homogeneous big initial rise, or (iii) either of them, usually b_2, failing to appear altogether.

Some specimens are shown in Fig. 2. Why in such cases the one or the other type of response is "switched on" remains obscure, but it makes

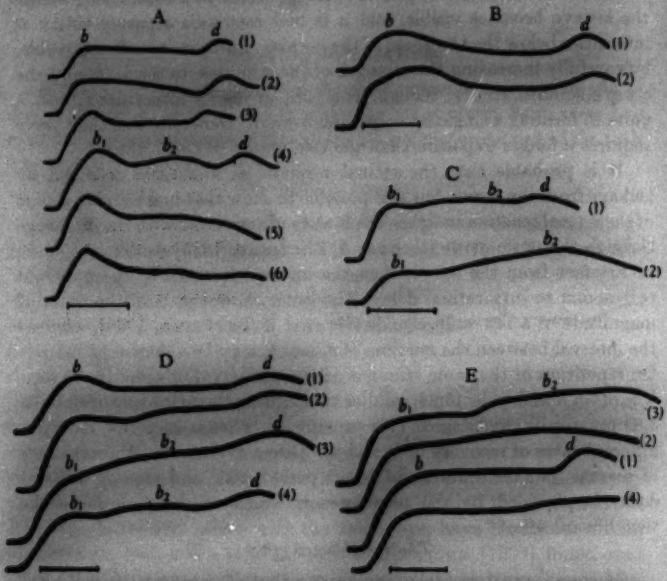

Fig. 2. Series of responses illustrating multiple b-wave and switchboard effect at low intensities. Figures against individual curves show order of the observations regardless of intervening exposures at other wave-lengths. Time in sec. A, Control at 0·510 μ showing various types of responses "switched on" during an experiment. Note b_2 appearing at (4), off-effect disappearing at (5). B, Two records at 0·510 μ. In (1) b-wave is 0·086 mV., in (2) the type of response suddenly has changed and b-wave now is 0·134 mV. Yet b_2 not clearly separable from b_1. C, Illustrates small b_2 at 0·510 μ, large b_2 at 0·570 μ. At 0·510 μ (upper response) b_1 is 0·200 mV., at 0·570 μ (lower response) b_1 is 0·105, b_2 0·152 mV. D, (1) and (2) are two responses at 0·510 μ, the latter one showing b_2: (3) and (4) at respectively 0·560 and 0·450 μ, the former one illustrating especially large b_2, not clearly separable from b_1. E, Four responses at 0·510 μ from experiment with typical switchboard effect changing b_2 and the off-effect. The initial phase, b_1, practically unchanged.

quantitative work near the threshold extremely difficult. The whole effect is most irregular; sometimes one type of response may be obtained for a long time, sometimes new combinations of b-waves are continuously appearing.

Only in one experiment did there seem to be a definite rule in the "switching" process. In this experiment a fast and a slow response alternated at both low and high intensities. The electroretinograms of Fig. 3 show results at the high intensity, used in the experiments collected in Fig. 4. A and B are two responses elicited by wave-length 0·420 μ, the pairs C and D, E and F were obtained with 0·600 and 0·510 μ respectively. In these three cases the stimuli were repeated at the standard interval of 3 min. Then, as two types of responses seemed to alternate, 0·450 μ was

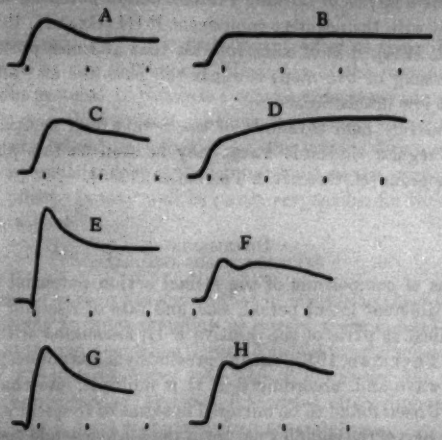

Fig. 3. Series of high intensity responses showing switchboard effect. A and B at 0·420 μ, C and D at 0·600 μ, E and F at 0·510 μ, G and H at 0·450 μ. Time in sec.

repeated six times at intervals of 3 min. The standard 1 sec. exposures were used. The two responses G and H alternated with perfect regularity and did so also at intervals from 1 to 4 min. It was further found that the slow response H at 0·450 μ also alternated with the fast response E at 0·510, but not so definitely with the fast response C at 0·600 μ. The eye seemed to be in excellent condition for several hours and there could be no question of fatigue.

The off-effect and the c-wave

With exposures of 3 sec. the off-effect is fairly regularly seen, even at very low intensities. In some eyes, however, it is absent. Very often the off-effect is present at the beginning of the experiment and absent at the

end of it. A switchboard effect like the one encountered with the *b*-waves also seems to be at work at "off". But as the off-effects are small it is difficult to be certain whether the "switching" process concerns different types of off-effects or not. "Off" and "on" often vary independently.

When light-adapted eyes have been allowed to dark-adapt and strong stimuli have been used, diphasic off-effects have often been observed [cf. also Smit, 1934], but these observations are better described in connexion with light adaptation [Granit & Wrede, 1936].

As on the analysis of the frog's retinal action potential the off-effect is connected with the negative component P III [Granit, 1933; Granit & Riddell, 1934], it is of some interest that at times purely negative responses, rising in two steps towards the base-line at "off", may be obtained at low intensities.

The relatively high threshold of the *c*-wave was mentioned above. Sometimes regular rhythmic waves may be seen on this phase of the electroretinogram [cf. Granit & Therman, 1935].

Discussion

In terms of components of the retinal action potential the various *b*-waves of different latent period, size, and rate of rise and decay have been described as parts of the positive P II, associated with excitation [Granit & Therman, 1935], though previously it has not been recognized that the *b*-wave and, accordingly, P II is multiple. As changes in the *b*-wave have been found to be mirrored in terms of frequency of impulses in the optic nerve, the complex nature of the *b*-wave would seem to imply that the discharge in the nerve consists of the summed activity of elements in which the frequency varies in rate of rise and decay.

In this respect our results and deductions, supported also by the work of Chaffee, Bovie & Hampson [1923] referred to above, are in complete agreement with Hartline's [1935] interesting observations on the discharge in single fibres of the frog's eye. In some fibres the frequency of the impulses rises and falls quickly, in others the discharge rises slowly and is better maintained. The off-effect also is more marked in some fibres, less developed in others, and some fibres only give an off-effect just as others only react to the onset of the stimulus.

The switchboard effect shows that there are a number of alternative pathways or couplings of pathways from the entrance station in the receptor to the optic nerve. This conclusion is independent of whether the switchboard effect is a normal or a pathological process. The mechanism

whereby sometimes one, sometimes another circuit is "switched on" can hardly be localized elsewhere than in the neurones between the receptor and the optic nerve. It is, of course, not necessary to represent the functional mosaic of different types of nerve responses or electrical component potentials as a pattern of stable units with different properties. The secret of the problem may lie in the switchboard effect being itself capable of providing different types of responses by suitably coupling units differing very little from one another. With inhibition also present in the retina [Granit & Therman, 1935; Hartline, 1935] the number of possibilities is legion.

The nearest analogy to the switchboard effect in a similar type of tissue seems to be the well-known "deviation of the response" in the central nervous system. In this case a constant electrical stimulus to the motor cortex may elicit various movement patterns. It is obvious that there are several visual phenomena, e.g. the various forms of contrast, which could be explained by a mechanism built on the principles of the switchboard effect. It may well be partly responsible for the lability of the visual threshold.

II. The measurements

The foregoing section has shown that in measuring the size of the *b*-wave we are using an index sampling, as it were, the sum total of positive components at the moment when they all combine in producing a maximal deflexion of the galvanometer. At higher intensities the *b*-wave is superficially homogeneous, at lower intensities often diphasic. In the latter case b_1 and b_2 have to be measured separately.

The *b*-wave however does not split up into components at a given level of intensity. The latter factor is important merely because it determines the size of the deflexion at the onset of the stimulus. Thus, when the *b*-wave diminishes towards the ends of the visible spectrum, it often becomes diphasic, even though in the optimal region of about 0·500 μ it is still homogeneous. Also, before the split is effected, b_1 and b_2 may combine so as to give relatively too big or too small responses in terms of p.c. of the value at 0·510 μ. Dependent upon the way in which b_1 and b_2 overlap the curves showing height of *b*-wave against wave-length could become too steep and narrow or too broad and flat. The switchboard effect, when present, may complicate matters, but it is generally recognizable and compensated for by a sufficient number of readings taken haphazard. A few series have been left out, as it proved to be impossible to decide what type of response to measure.

The curves plotted on size of *b*-wave against wave-length will be compared with the absorption curve for visual purple [Köttgen & Abelsdorff, 1896; Trendelenburg, 1904]. As V.P. standard will be used a curve, kindly sent to us by Dr R. J. Lythgoe and obtained by the method used by Bayliss, Lythgoe & Tansley [1936] in their recent work with eyes of various sea fishes. The curve is based on observations with V.P. of six *Esculentas*.

Separation of rods and cones

Fig. 4 shows the averaged results at the high intensity, based on about 500 observations with twenty frogs. As a measure of the strength of the stimulus we have used the average of all the maximal *b*-waves at 0·510 μ

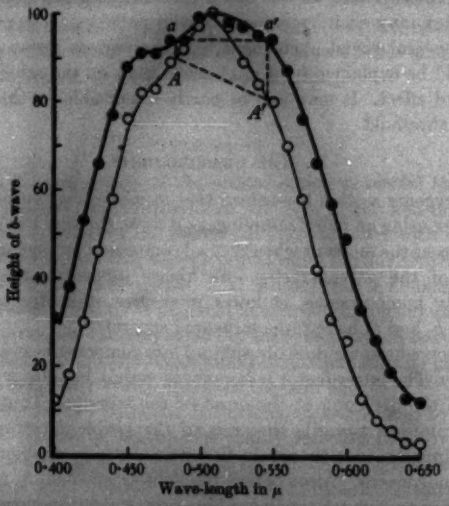

Fig. 4. Height and rate of rise of *b*-wave in p.c. of value at 0·510 μ, plotted against wave-length. Dots = height, circles = rate of rise. High intensity. Winter frogs.

in each series. This value here is 0·686 mV. The outer curve shows height of *b*-wave calculated in p.c. of the value at 0·510 μ. The inner curve shows the average rate of rise in p.c. of the value at 0·510 μ. Rate of rise is defined by the height of *b*-wave divided by the time during which it has risen to its top value. The average maximal rate of rise at 0·510 μ is

$2{\cdot}96 \times 10^{-2}$ mV./msec. The band of maximal responses is very broad, values between 0·450 and 0·560 μ differing from one another by less than 12 p.c. A hump is found at 0·460 μ. In the individual curves its locus has varied between 0·450 and 0·470 μ.

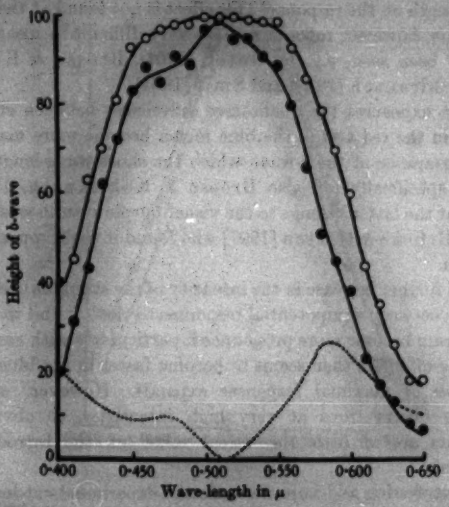

Fig. 5. Height of b-wave in percentage of value at 0·510 against wave-length. Dots = separately averaged values above the common averages shown in Fig. 4. Circles = averages of values below the common average.

The average curve for size of b-wave has been split up in Fig. 5, in which the values above and those below the average have been separately averaged. The dotted curve below shows the difference between them. The hump at 0·460 μ seems to be confined to the lower curve; the band of maximal responses in the upper curve shows that there have been eyes in which the response has been practically maximal between 0·450 and 0·560 μ. In contradistinction to the average curves obtained at lower intensities these are asymmetrical around 0·507 μ. The difference between low and high responses is maximal around 0·580 μ.

If in Fig. 4 equipotential points a and a' be chosen on either side of the maximum of the average curve, the line AA' shows the corresponding

values on the inner curve for rate of rise. Obviously the *b*-wave on the red side rises more slowly to a given potential than does the *b*-wave on the blue side of the spectrum. Therefore the elements or the combination of elements giving potential *a* are not identical with those giving the same potential (*a'*) on the other side of the maximum. There is a specific effect of wave-length on the response. This effect is not found at the base of the curve where, however, rates of rise are very difficult to measure. It has previously been seen, e.g. by Gotch [1904], Brossa & Kohlrausch [1913], Kohlrausch [1918] and Smit [1934].

At long exposures the qualitative differences between equipotential responses in the red and in the blue region become more marked owing to the appearance of the *c*-wave which the short wave-lengths seem to stimulate specifically (cf. also Brossa & Kohlrausch, 1913; Smit, 1934). That the latter belongs to the visual purple system was shown long ago by v. Brücke & Garten [1907] who found it to disappear after light adaptation.

After a further increase in the intensity of the stimulus the qualitative differences between equipotential responses to the left and to the right of the maximum become more pronounced, particularly with respect to rate of rise. The off-effect then seems to become faster in the blue region and the plateau of maximal responses expands. However, we have no systematic observations at very high intensities, as obviously such experiments are of little theoretical value (cf. the Introduction and last section).

More interesting and important are the experiments at lower intensities. In Fig. 6 the average maximal *b*-wave at 0·510μ is 0·272 mV. and the number of observations 392 (fourteen frogs). The spectrum is of feeble intensity but is still coloured to the dark adapted human eye. Below 50–60 p.c. of the maximum the *b*-wave is diphasic but b_1 alone has been plotted for the whole spectrum. In p.c. of the value at 0·510μ, b_2, which is superimposed upon what remains of b_1 (cf. Fig. 1), is larger than b_1 so that a curve plotted on b_2 would broaden downwards as shown by the values for b_2 towards the red side. The inner curve shows rate of rise of the *b*-wave, the average maximal rate of rise being $4{\cdot}9 \times 10^{-4}$ mV./msec.

The curves tend to be symmetrical, and are considerably narrower and steeper than the high intensity curves. As the value for 0·520μ is below the value for 0·500μ, the maximum probably is between 0·510 and 0·500μ. There are no qualitative differences between equipotential responses in the various regions of the spectrum. All measurements at or

below this intensity show that the rate of rise is a function of the size of the *b*-wave and is unaffected by wave-length as such. The eye now reacts like the rod eye of the rat [Graham & Riggs, 1935].

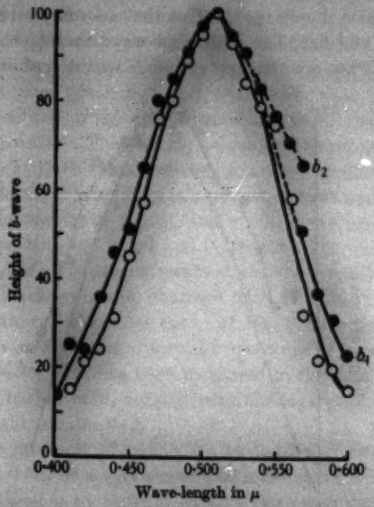

Fig. 6. Height and rate of rise plotted as in Fig. 4. Dots = height, circles = rate of rise. Low intensity. Winter frogs. Note: in this group of experiments b_2 appeared fairly regularly in the red, less regularly in the violet; in the red critical region where b_1 and b_2 separate. Further towards the red only b_1 plotted. In the violet only b_1 plotted.

The low intensity response

Though now we are dealing with a simple type of reaction in which a variation in wave-length influences size alone and not shape of the response, there are other complications left which deserve to be studied in detail. The difficulties encountered in exploring this region are clearly shown by Fig. 7 in which b_1 and b_2 have been plotted separately in terms of p.c. of the value at 0·510 μ. As b_2 does not occur regularly, only 165 observations are available. The average curve for b_1 (801 observations) is shown in the same figure. The irregularities in the form of the curve for b_2 are due not only to the small number of observations, but also and, perhaps, chiefly to the variations in b_2 itself. As often is the case with a

well-developed diphasic response at low intensities, the switchboard effect was a serious disturbance, occurring in four of the nine experiments concerned.

Apart from showing how difficult it is to obtain accurate results with b_2, the figure also illustrates the fact that curves plotted on b_2 tend to become broad and flat. The multiple b-wave therefore is an important source of error when comparisons are made with the absorption curve for

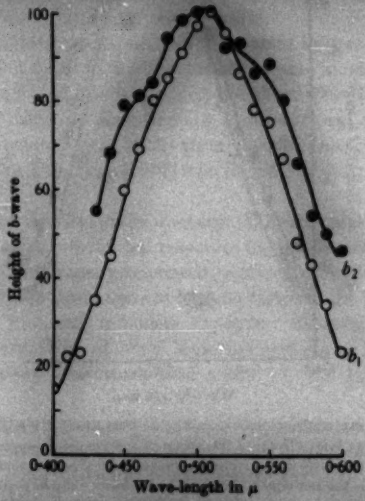

Fig. 7. Height of b_1 (circles) and b_2 (dots) plotted as in Fig. 4. Low intensity. The curve for b_1 average of 801 observations with twenty-seven frogs; the curve for b_2 165 observations unevenly distributed over the same material.

visual purple. We have to reckon with the possibility that b_2 also influences the curve for b_1 in cases where two or several b-waves overlap so as *not* to be separable. But as this effect is independent of wave-length as such,[1] nothing but symmetrical curves, broader or narrower, can be obtained from a large body of data if the average symmetrical curve of Fig. 7 is transformed by disturbances arising from the multiple nature of the b-wave or the switchboard effect.

[1] Actually we have had series in which b_2 has been much more definite *either* in the red (cf. Fig. 6) *or* in the violet. But such differences disappear with a large material of averaged results.

The average curve for b_1, drawn in Fig. 7, to some extent misrepresents our actual findings. In the individual curves behind the average some values may be too low or too high, simply because of the ordinary random distribution of errors. But, considering the nature of the switchboard effect, some values may also be too low or too high because they belong to different curves. In addition the average curve is a sum of individual curves differing typically among themselves. All these factors are taken account of, and, at the same time, the advantages of averaging preserved, if the values below and those above the average are averaged separately. This has been done for the results presented in Fig. 8 and Table I. In this final presentation of our measurements at low intensities two levels of intensities (slit openings) have been used, the one employed for the results of Fig. 6, and a still lower intensity eliciting maximal *b*-waves between 0·200 and 0·100 mV. The average maximal *b*-wave at 0·510 μ of all the experiments at the low intensities is 0·20 mV. The number of readings is 801, obtained with 27 frogs.

The circles around the inner curve of Fig. 8 have been taken from Dr Lythgoe's curve for the absorption spectrum of visual purple. The absorption curve for V.P. has been measured in terms of densities for a solution of V.P. in digitonin, density being the logarithm of the fraction, light incident/light transmitted.

Through the two sets of values we have plotted two curves, symmetrical around 0·507 as a maximum. The inner curve is better fitted by our own data as well as by those of Dr Lythgoe with visual purple. Yet the two sets of data do not agree as well as might have been expected. The outer curve seems to be more influenced by factors tending to produce irregularities (see the mean variations, Table I). However, both types of curves are typically obtained with individual eyes. As we have averaged thirty observations with each preparation, there can be no doubt about the fact that some eyes give broad, others narrow, curves. But there is also a minor number of eyes which equally definitely give asymmetrical curves or, at least, curves shifted towards the red or the blue side of the spectrum, corresponding roughly to inner left, and outer right leg of the averaged curves combined, or vice versa. Thus four types of response curves may be obtained, exemplified by combining the four legs of the average curves in the four possible ways. In some cases the spectrum gradually has become shortened in the region of red towards the end of the experiment, in other cases the switchboard effect has produced a sudden change in the curve. But though such results indicate causes of lability, most eyes have been fairly stable. The mean variations show

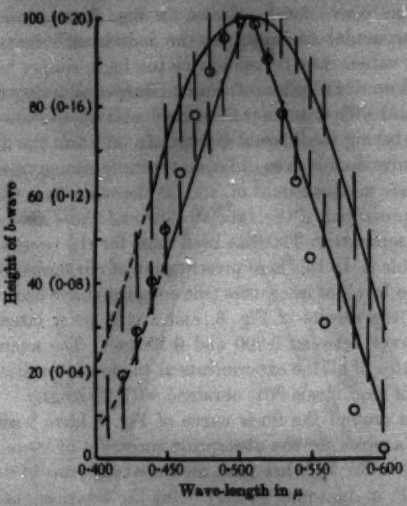

Fig. 8. Height of *b*-wave in mV. and in p.c. of value at 0·510 μ against wave-length. Inner and outer curve drawn symmetrically through respectively low and high values separately averaged as explained in text. Same material of twenty-seven winter and spring frogs as in Fig. 7 (see also Table I). Values marked by vertical lines giving mean variations doubled. The curves are drawn with broken lines through the region in which energy of spectrum not corrected. Circles = absorption of visual purple in digitonin; values obtained from Dr R. J. Lythgoe.

TABLE I

Wave-length in mμ	400	410	420	430	440	450	460	470	480	490	500
Average height of large *b*-waves	22	33	33	44	59	72	80	87	94	97	100
Mean variation	7·0	5·0	3·6	7·6	8·9	9·1	7·8	5·7	7·0	4·3	1·1
Average height of small *b*-waves	8	12	13	26	39	48	57	72	75	82	94
Mean variation	6·7	7·0	6·1	4·2	6·6	6·2	5·7	4·4	6·1	4·0	2·9

Wave-length in mμ	510	520	530	540	550	560	570	580	590	600
Average height of large *b*-waves	100	99	95	88	85	79	58	55	43	33
Mean variation	—	2·5	4·8	4·4	5·8	5·8	4·9	10·3	9·1	6·2
Average height of small *b*-waves	100	90	76	68	66	54	38	31	25	12
Mean variation	—	3·3	5·0	5·0	3·9	5·8	4·4	5·5	3·6	5·6

the individual results to have been particularly labile between 0·440 and 0·460 and about 0·580 μ.

The off-effect and the c-wave

The off-effect at low intensities is rather too small to be accurately measured and often is very irregular. In three experiments it has been exceptionally well developed and regular and for them the off-effects

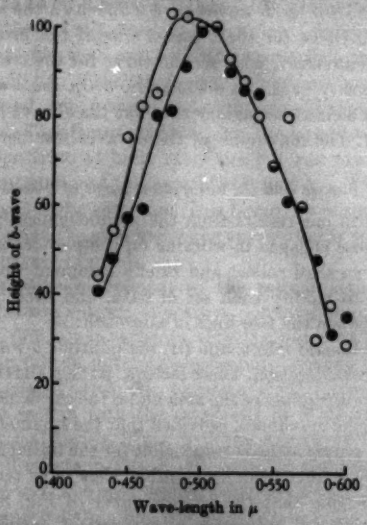

Fig. 9. Height of off-effect (circles) and *b*-wave (dots) plotted against wave-length as in Fig. 4. Low intensity.

have been averaged and plotted in Fig. 9 in terms of p.c. of the value at 0·510 μ. The corresponding *b*-waves are given in the same figure, similarly averaged. The mean values for the maximal *b*-waves and off-effects at 0·510 μ were respectively 0·174 and 0·068 mV. The number of observations was 50.

The curve for the off-effect is markedly asymmetrical, compared with the curve for the *b*-wave. The large off-effects in the blue region were seen in all the three experiments. This does not necessarily mean that the

curve for the off-effects generally is asymmetrical and maximal about 0·490 μ. There have been cases in which the off-effect seems to have followed the curve of the corresponding *b*-waves. On the other hand, we have seen *b*-waves in other eyes asymmetrical and expanded towards the short wave-lengths. We can therefore only conclude that *b*-waves and off-effects show variations within the same limits but may do so independently of one another.

The *c*-wave has only been measured once, when at the higher intensity (see Fig. 4), despite the short exposure of 1 sec., it nevertheless was very large. Thus at 0·510 μ the *b*-waves gave 0·520 mV., the *c*-wave 1·50 mV. In this case the curve for the *b*-waves was of the broad type always obtained at this intensity, whereas the curve for the *c*-waves was of the narrow low intensity type. At 0·430 and 0·590 μ the *c*-wave hardly was measurable as a definite secondary rise, but the *b*-wave fell on the curve shown in Fig. 4. The maximum of the *c*-waves was between 0·500 and 0·510 μ.

Size of b-wave and the absorption curve of visual purple

A discussion of our results from the photochemical point of view is better left to those engaged in working on the photochemistry of visual purple. Below we shall collect and briefly comment upon a number of factors tending to modify each set of data, and thereby to invalidate comparisons between the two lines of approach:

(i) The switchboard effect, and (ii) the multiple *b*-wave have already been given due consideration. These factors, with a sufficient number of data, should only influence the area under the curve, not the symmetry and the locus of the maximum, provided that the symmetrical absorption curve for visual purple alone is responsible for the initial reaction to light. When these effects have been measurable, they have tended to broaden the curve, suggesting that the narrow curve of Fig. 8, best fitted by Dr Lythgoe's values, represents a theoretical optimum among the possible combinations of *b*-waves.

(iii) The intensity of the stimulus likewise has been considered inasmuch as too strong stimuli may activate high threshold receptors lacking visual purple (cones). These receptors alone give a symmetrical curve with a maximum about 0·560 μ and 50 p.c. of the maximum about 0·490 and 0·640 μ [Granit & Wrede, 1936]. At 0·450 μ values of about 25–30 p.c. are obtained. The cones no doubt account for the specific differences with respect to wave-length found at higher intensities. To prevent what seems to be a common misunderstanding, it is necessary to point out that in this case the specific differences with respect to wave-length prove nothing

about the presence of receptors selectively sensitive to colour. They can be fully explained by the shift in the curve (the Purkinje shift), leading to various combinations of rod and cone *b*-waves, as well as by the absence of *c*-waves in light-adapted frogs' eyes. The faster rate of rise in the blue region may be due simply to rods and cones both adding to the rate of rise in this part of the spectrum, whereas towards the red and the violet ends one type of receptor alone predominates.

(iv) But intensity of the stimulus may also come in as a source of error tending to make the curve for the *b*-waves too broad, if, as seems probable [cf. Graham, Kemp & Riggs, 1935], there be a definite limit for the production of potential in the retina. Supermaximal stimuli around 0·500μ would then cause maximal potentials, but so would also stimuli at, say, 50 p.c. of the maximum. Dependent upon the amount of overlap of rod and cone *b*-waves the curves would then be symmetrical or asymmetrical. This factor could explain the large *b*-waves in the blue region of the spectrum at high intensities but not the fact that many eyes give similar curves at the lowest intensity.

(v) Stray light in the spectrum. This factor, by raising the low values, would tend to widen the area under the curves, but it cannot explain the four types of curves obtained.

(vi) The negative component of the retinal action potential, by neutralizing a certain amount of positive potential, may be an important source of error, difficult to estimate. Having seen that the off-effect and the *b*-wave may fall on different curves and knowing the former to be intimately associated with the negative P III [Granit & Riddell, 1934], we must reckon with the possibility of P III also being able to cause asymmetrical curves. Thus, with the negative P III falling on the curve for the off-effects in Fig. 9, the curve for the *b*-waves would shift towards the red end. The only negative deflexions measured actually had their maximum in 0·490μ. But this explanation of the asymmetry does not remove the necessity of finding some substance other than visual purple or a modification of visual purple in order to explain the presence of asymmetrical curves. It merely means that this substance may activate the negative instead of the positive component and that instead of two substances only one substance need be evoked to account for the two asymmetrical curves.

(vii) Selective absorption in the media of the eye should be mentioned though in the few cases, when opened bulbs have been used, it has not been noted. This factor can hardly be of any significance compared with other sources of error.

(viii) The concentration of visual purple in living rods may be high, in which case the absorption of light would not be proportional to the density of the solutions at various wave-lengths. As Trendelenburg [1904] has pointed out, this would tend to make the effective absorption curve for visual purple too narrow.

(ix) The photochemical work suffers from the disadvantage of necessitating the data for absorption of visual purple to be obtained as a difference between absorption in an unbleached and in a bleached solution. This means that by-products in the formation and decomposition of visual purple, which well might be photochemically and physiologically active, do not necessarily affect the absorption curve. If relatively more stable than visual purple, they would remain unmodified in the bleached solution [Garten, 1907]. Progressive differences in the density between bleached and unbleached solutions may also prove difficult to compensate for, as found by Bayliss, Lythgoe & Tansley [1936].

Recent work by Hosoya & Bayerl [1933] and Hosoya [1934], confirmed by Hecht & Chase [1934], Bayliss, Lythgoe & Tansley [1936], and by Wald [1935] from a different angle, seems definitely to have established the reality of visual yellow, advocated long ago by Kühne [1879], Garten [1907], and others. The presence of this blue absorbing substance in varying amounts, coupled with the assumption that it is physiologically active, satisfactorily explains why in some animals the curve for the *b*-wave extends too far towards the violet end to fit the curve for pure visual purple. Smit [1934] also mentions that he has recorded very large responses at 0·400μ [cf. also Chaffee & Hampson, 1924]. The hump at 0·460μ at high intensities and the large mean variations in this region at low intensities can perhaps be similarly explained [cf. in particular Hecht & Chase, 1934].

Köttgen & Abelsdorff [1896] found two types of visual purple, of which the second type with a maximum absorption at 0·540μ was seen in fishes. Bayliss, Lythgoe & Tansley [1936] have described several new forms of V.P. with different absorption maxima which, however, seem to be typical of certain species of fish. They can also be extracted by digitonin and therefore cannot account for the difference between our results and those of Dr Lythgoe with the same experimental animal. In the blue and the violet the values of Dr Lythgoe fall between our two curves but towards the red our readings are relatively too big (see Fig. 8). There is the further difference in the results that our material definitely shows different types of response curves whereas, according to Dr Lythgoe, the absorption curves for V.P. are constant within a few

p.c. By suitably selecting the series to be averaged we could obtain curves fitting the absorption curve for visual purple fairly precisely, particularly in the violet, but this result is not obtained with a sufficiently large material. Hecht [1924] points out that the human "visibility curves" of dark-adapted eyes also are shifted towards the red side compared with the absorption curve for V.P.

In order to explain the discrepancy, we have to choose between the possibilities mentioned above (see especially (vi) and (ix)) and the assumption that there are low threshold elements selectively sensitive to the long wave-lengths, e.g. the "green rods" of Schwalbe. The former alternative is suggested by the lability of the response curves and the possibility of visual yellow being formed at different rates in the elements responsible for the various component potentials, the latter by the fact that large responses in the red seem to have been generally obtained, not only by ourselves but also by Himstedt & Nagel [1901], Piper [1904], Brossa & Kohlrausch [1913] and Chaffee & Hampson [1924].

Summary

The *b*-wave of the electroretinogram of dark-adapted frogs' eyes has been recorded with string galvanometer and directly coupled amplifier. Monochromatic stimuli 0·010μ apart in an equal energy spectrum have been used. The results are described in a section on "general observations" and in one concerned with "the measurements" of height and rate of rise of the *b*-wave.

In the former section it is shown that the *b*-wave is multiple and consists of several components of different latent period, size, and rate of rise and decay. At low intensities the various components of the *b*-wave may combine differently in response to a constant stimulus. This lability of the response has been termed "the switchboard effect".

In the latter section the measurements at high and low intensities are separately presented. The *b*-waves have been plotted against wave-length in terms of p.c. of the value at 0·510μ.

At high intensities broad, often asymmetrical curves with expanded maxima are obtained, and the *b*-waves show specific differences with respect to wave-length.

At low intensities most eyes give symmetrical curves around a maximum of about 0·507μ. They are either broad or narrow (see Fig. 8). The *b*-waves do *not* show specific differences with respect to wave-length.

Some eyes give asymmetrical curves or curves shifted slightly towards the red or the violet end of the spectrum. In some cases the maxima of *b*-wave and off-effect fall on different curves.

The results are compared with the absorption spectrum for visual purple (Lythgoe), and various sources of error tending to invalidate such comparisons are discussed. It is shown that visual purple cannot alone mediate the responses, obtained from frogs' eyes at low intensities. Other alternatives are pointed out. The specific differences with respect to wave-length at high intensities are held to be due to cones.

We are indebted to Mr K. Holmberg of the Physical Laboratory of this University for valuable suggestions.

One of us (R. G.) also wishes to acknowledge with gratitude a grant from the Rockefeller Foundation enabling him to obtain the monochromator used in this work, and a grant from the Societas Scientiarum Fennica covering part of the expenses connected with the experiments.

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EFFECTS ON THE KNEE JERK OF STIMULATION OF THE CENTRAL END OF THE VAGUS AND OF VARIOUS CHANGES IN THE CIRCULATION AND RESPIRATION

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(*Received* 2 *September* 1936)

A number of workers have presented evidence that afferent autonomic impulses may produce reflex effects on skeletal muscle. Miller [1924] and Miller & Waud [1925] found that stimulation of the central end of mesenteric nerves produced contraction of the abdominal wall and of certain muscles of the limbs. Later observers showed that stimulation of the sino-aortic nerves may reflexly modify muscle tone and other skeletal activities. According to Danielopolu [1927] stimulation of the sinus nerves increases the tone of the intercostal and other chest muscles. Tournade & Malméjac [1929] found that the shivering movements normally induced by exposure to cold were inhibited by sinus nerve stimulation. Koch [1931] and later Schweitzer [1934, 1935] showed that the spontaneous movements of anæsthetized animals were inhibited reflexly by raising the pressure in the isolated carotid sinuses. Extending this work Koch [1932] found that raising the carotid sinus pressure induced general loss of muscle tone and a condition resembling sleep in unanæsthetized dogs; on lowering the pressure again tone and consciousness returned. King *et al.* [1931] noted that central vagus stimulation may depress the knee jerk in anæsthetized animals. Spychala [1932, 1933] obtained the same result on raising carotid sinus pressure and found at the same time a decrease in the frequency of the action currents in the resting quadriceps muscle. Strughold [1929], who observed respiratory variations in the extent of the knee jerk, and Johnson & Luckhardt [1927], who noted that the knee jerk was diminished when the intrapulmonary pressure was markedly raised, attributed their results in part to the effect of afferent vagal impulses. The latter workers

found that stimulation of the central end of the vagus diminished the knee jerk even when the blood pressure was raised or unchanged.

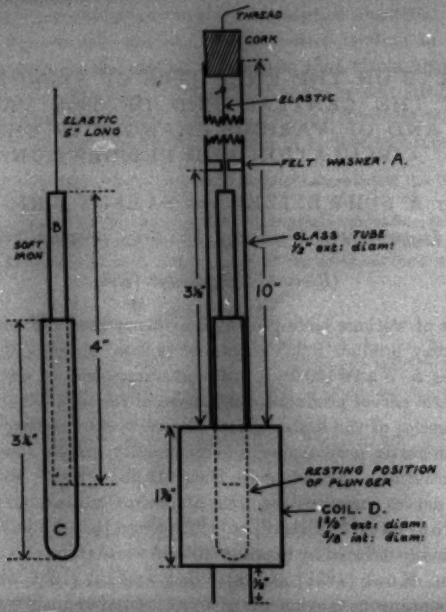

Fig. 1. Diagram of apparatus for automatically eliciting the knee jerk. The apparatus consists of a short cylindrical rod (*B*) of soft iron suspended by an elastic thread inside a glass tube arranged vertically. The lower end of the iron rod is covered by a cylinder of bakelite material (*C*) (top of fountain pen) and the covered rod is just freely movable along the axis of the tube. A coil (*D*) of about 800 turns of enamelled copper wire (size s.w.g. No. 24 and of resistance about 4·5 ohms) is wound on a bobbin of the dimensions shown and fitted over the glass tube, so that the iron rod is half inside the coil. When the current from a 4-volt battery is passed through the coil the iron rod is attracted downwards rapidly by magnetic action and the bakelite end piece is utilized as a hammer-head to strike the tendon. The small piece of felt (*A*) shown in the diagram inside the tube above the iron rod is placed there to damp out the free oscillations which occur on the return of the rod when the current circuit is opened. The circuit is interrupted at the appropriate rate, usually once in 5 or 10 sec.

We have re-examined the effects of central vagus stimulation on skeletal muscle activity using the knee jerk as our type reflex. In the

course of our study we also investigated the effects on the knee jerk of (i) lowering the blood pressure by other methods, (ii) acute anæmia of the central nervous system, (iii) anoxia, (iv) changes in the carbon dioxide pressure in the blood, and (v) various doses of histamine and cyanide.

Methods

Cats were used exclusively, anæsthetized with chloralose (0·06–0·08 g. per kg. body weight). The knee jerk was recorded in the following manner. The limb was suitably fixed by means of drills in the femur and ischial tuberosity and the leg was flexed at the knee joint. The patellar tendon was regularly tapped at intervals of 5 or 10 sec. by means of an electrically operated automatic hammer, the construction and mode of action of which is shown in Fig. 1. The extension of the knee was recorded by means of a thread which was fixed to the lower end of the tibia and connected over pulleys to a Sherrington torsion lever myograph. In some experiments the foot was supported and in others the leg hung freely, so that the contraction of the quadriceps was either after-loaded or free-weighted. In the opposite leg the response of the quadriceps to stimulation of its motor nerve was frequently recorded simultaneously by means of another torsion lever myograph. The femoral nerve was exposed in the abdomen and stimulated submaximally with a fluid electrode of the Collison type at the same rate as the knee jerk was being elicited. The tension obtained during the knee jerk varied with different preparations but was usually quite low, and of the order of 100–300 g. The knee jerk was usually recorded on the right side and the central end of either the left or the right vagus nerve was stimulated. Spread of current to the medulla was prevented by carefully dissecting the vagus nerve for 5 cm. above the point stimulated and fixing the nerve on electrodes that were held at least 1 in. above the neck tissues.

Results

Stimulation of the central end of the cut vagus, as has been shown by other workers [Johnson & Luckhardt, 1927; King *et al.* 1931], produces a temporary depression or abolition of the knee jerk (Fig. 2) which may be associated with a decrease in quadriceps tone. The inhibition sets in within a few seconds of the onset of stimulation and commonly the next jerk shows diminished tension. The depression of the jerk may precede the onset of the fall of blood pressure. As stimulation is continued the inhibition becomes progressively deeper, a

phenomenon analogous to the inhibitory recruitment of Sherrington. Recovery is usually gradual. Increase in the strength of afferent vagus stimulation increases the extent of the inhibition and prolongs the time necessary for recovery (Fig. 2). The inhibition may outlast by many minutes the return of the blood pressure to normal. In some instances the inhibition of the jerk becomes greater when afferent vagal stimulation is discontinued (Figs. 3, 4). This phenomenon is analogous to the inhibitory after-discharge of Sherrington, and is sometimes associated with an after-fall of the blood pressure. In the case of skeletal muscle reflexes such a result is usually interpreted to indicate that the afferent nerve concerned contains a mixture of pressor and depressor fibres, the effect of the former dying down more rapidly so that the effect of the latter becomes revealed mainly after stimulation has ceased.

Fig. 2. Cat. Chloralose (0·08 g. per kg.). Records from above downwards are knee jerk, contraction of quadriceps stimulated through its motor nerve, blood pressure, time in 30 sec. and signal line. At the times indicated by the signal, the central end of the left vagus was stimulated, (i) coil at 12, (ii) coil at 9.

In animals under light chloralose anæsthesia with exaggerated reflex excitability manifesting itself by irregular responses to patellar stimulation and considerable spontaneous general activity, it is found that central vagus stimulation not only diminishes the knee jerk but also depresses general muscular activity. Both the knee jerk and the general excitability return at about the same rate when stimulation is stopped (Fig. 3).

It can be readily shown that the effects on the knee jerk recorded above are in no way due to alterations in the condition of the quadriceps muscle itself. Thus in Fig. 2 there is no diminution in the response of the quadriceps to submaximal stimulation of its motor nerve during a period of central vagus stimulation, though the knee jerk was simultaneously markedly inhibited on the other side.

The effects described are not modified by section of the opposite vagus or by denervation of the carotid sinuses.

Effect of atropine

After the injection of atropine in sufficient dosage to paralyse the peripheral vagal terminals in the heart the inhibition of the knee jerk produced by central vagal stimulation is unaffected.

Fig. 3.

Fig. 4.

Fig. 3. Cat. Chloralose (0·06 g. per kg.). Both vagi cut and sinuses denervated. Records from above downwards are knee jerk, blood pressure, signal line and time in 30 sec. Stimulate the central end of left vagus, coil at 9.

Fig. 4. Cat. Chloralose (0·08 g. per kg.). Both vagi cut. Records from above downwards are respiration, knee jerk, blood pressure, signal line and time in 30 sec. (i) Inject 0·05 mg. histamine. (ii) Stimulate central end of left vagus.

Relation to respiratory changes

The inhibition of the knee jerk is independent of the nature of the respiratory response and occurs equally well when there is apnœa, no alteration in the breathing or increase in respiratory tone and movements.

Relation to blood pressure changes

It can be shown in many ways that the inhibition of the knee jerk is not dependent on the changes in the blood pressure.

(i) The knee jerk may be more depressed in experiments when the blood pressure fall is small than when it is large.

(ii) Following section of the opposite vagus or atropinization, stronger stimulation of the central vagus produces a smaller fall of blood pressure than previously, but gives rise to a greater inhibition of the knee jerk.

(iii) In animals with the vaso-sensory nerves divided the blood pressure may show spontaneous falls without any change in the knee jerk, while a fall of similar magnitude during central vagal stimulation gives the usual depression of the knee jerk (Fig. 3).

(iv) Not infrequently the inhibition of the knee jerk may become more marked as the blood pressure is returning to normal (Fig. 4).

(v) When a compensator device is employed [Wright, 1930] to minimize the fall of blood pressure the effect of central vagal stimulation on the knee jerk is unaffected.

(vi) Following the injection of small doses of ergotamine which abolishes the depressor effect on the blood pressure of central vagus stimulation [Rothlin, 1923; Wright, 1930], the inhibition of the knee jerk can be produced as readily as before the administration of the drug.

(vii) In agreement with Johnson & Luckhardt [1927] we find that inhibition of the knee jerk may occur when the blood pressure is raised by central vagus stimulation.

(viii) A sudden marked lowering of the blood pressure, e.g. from 130 to 60 mm., by means of hæmorrhage in the denervated animal has no depressor effect on the knee jerk. If the blood pressure is gradually lowered by means of repeated small hæmorrhages, the knee jerk sometimes becomes progressively enhanced.

(ix) Small doses of histamine which produce a fall of blood pressure as sudden and greater than that resulting from central vagus stimulation produce a small and very transient degree of depression of the knee jerk (Fig. 4).

It may be concluded from all these observations that central vagus stimulation depresses the knee jerk by an action on the central nervous system which is independent of the coincident circulatory or respiratory changes.

Action of eserine

Dikshit [1934] found that the introduction of minute doses of acetylcholine into the cerebral ventricles of the cat inhibited respiration in a manner resembling that produced by central vagus stimulation. Neither effect was abolished by atropine or enhanced by eserine. In occasional experiments he found an increase in the acetylcholine content of the

cerebro-spinal fluid after central vagus stimulation. Feldberg & Schriever [1936] pointed out that without previous injection of eserine it is unlikely that any acetylcholine formed will escape destruction before entering the cerebro-spinal fluid. They found that eserine increases the acetylcholine content of the cerebro-spinal fluid, but that central vagus stimulation in eserinized animals has no such effect. We have observed [Schweitzer & Wright, 1936] that the intravenous injection of acetylcholine in animals with or without previous treatment with eserine usually decreases the knee jerk, so the possibility has to be considered that central vagus stimulation may act on the knee jerk through such a central transmitter. We have found, however, that the vagal inhibition of the knee jerk is entirely unaffected by the previous administration of eserine.

These results with eserine and atropine are in agreement with those reported by Dikshit [1934] with reference to central vagus action on respiration, but do not support the view that acetylcholine is concerned with either reaction.[1]

Action of histamine

The mode of action of histamine was further examined. Doses up to 0·2 mg. have no effect on the response of the quadriceps to stimulation of its motor nerve, although the knee jerk is simultaneously depressed. An increase in the dose of histamine employed, e.g. from 0·05 to 0·2 mg., may not further increase the fall of blood pressure but may intensify and prolong the inhibition of the jerk (Fig. 5). This suggests that histamine acts directly by depressing the nerve centres.

When the fall of blood pressure, produced by histamine, is great and sudden there may be a short initial stimulation of the knee jerk (Fig. 5) followed by a prolonged depression. Thus in one experiment in which the blood pressure was lowered by histamine from 120 to 10 mm. and remained at that level for 3 min., there was an initial stimulation of the knee jerk followed by its complete disappearance for 7 min. Experiments reported in a subsequent section show that this effect is identical with and probably largely due to acute anæmia of the central nervous system. But as has been pointed out, quite apart from this complicating action of anæmia, histamine has a direct though slight depressant action on the nerve centres.

[1] *Note added in proof.* Experiments to be reported later have shown however that eserine has a "strychnine-like" action on the spinal cord causing an increase in the knee jerk, and tending to mask any potentiation that might occur of the central inhibition set up by afferent vagus stimulation.

Effect of peripheral anæmia

Obstruction of the lowest part of the abdominal aorta and inferior vena cava for periods of 10–15 min. may not depress the knee jerk at all or only to a trivial extent (Fig. 6). It should be borne in mind that during this period the work performed by the quadriceps is equivalent only to maintaining a tension of 12 kg. for less than 1 sec., so that the call on the energy reserves of the muscle is very small.

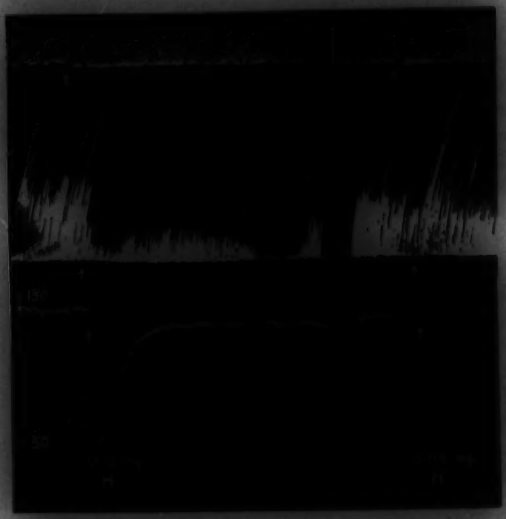

Fig. 5. Cat. Chloralose (0·08 g. per kg.). Both vagi cut. Records from above downwards are respiration, knee jerk, blood pressure, signal line and time in 30 sec. Inject (i) 0·2 mg. and (ii) 0·05 mg. histamine. Note the slight transient initial stimulation of the knee jerk during the period of rapid fall of the blood pressure.

Effects of acute complete anæmia of central nervous system

Clamping the arch of the aorta at its origin from the heart produces, as found by previous workers [see Sherrington, 1900], an initial stimulation of the knee jerk (Fig. 6). After ischæmia has been maintained for a variable length of time (3–7 min. in different experiments), the knee jerk rapidly declines and completely disappears. Restoration of the circulation causes recovery to set in after a latent period which is again

variable but has in some of our experiments been as long as 7 min. The knee jerk then progressively returns to its initial amplitude.

It seems that once the excitability of the anterior horn cells has been abolished by acute anæmia, a considerable period of time may have to elapse before restoration of function takes place. As has been suggested, the transient increase in the knee jerk commonly seen when the blood pressure is rapidly lowered to a large extent is probably produced in a similar way. In our experience the critical level of arterial blood pressure necessary to produce anæmic central stimulating effects is about

Fig. 6. Cat. Chloralose (0·07 g. per kg.). Records from above downwards are knee jerk, blood pressure from left carotid and time in min. In (*A*), clamp the lowest part of the abdominal aorta and inferior vena cava. In (*B*), clamp the aorta at its origin from the heart. In (*C*), same as (*B*) for a shorter period.

30–40 mm., though occasionally slight transient stimulation is observable with blood pressures of 60 mm. if the fall has been sufficiently considerable in extent, e.g. about 100 mm. (cf. Fig. 5).

The experiments have been repeated in animals with the spinal cord divided in the mid-thoracic region. In such preparations occlusion of the aorta at its origin from the heart produces effects on the knee jerk in no way different from those observed in animals with intact central nervous system. It may, therefore, be concluded that the effects produced by acute anæmia are due to a direct action on the lower spinal centres.

Attention must be drawn to the difference between the response of the respiratory and spinal centres to acute anæmia. When complete anæmia of the respiratory centre is produced in animals under chloralose anæsthesia by occluding the carotid and vertebral arteries, there is a rapid failure of respiration without any initial stimulation.

It is interesting to note that during acute cerebral anæmia the knee jerk may also be substantially depressed without initial stimulation. Montgomery & Luckhardt [1930] found on markedly raising the intracranial pressure and so producing cerebral anæmia that the knee jerk was promptly depressed; but when the experiment was repeated following division of the spinal cord above the knee jerk centre, the knee jerk was not affected until the respiratory and vaso-motor centres were paralysed. On the other hand following transection of the spinal cord, increase in the intraspinal pressure, to produce local spinal anæmia resulted (in agreement with our findings) in a temporary marked increase in the jerk followed by its abolition. It may be concluded, therefore, that cerebral anæmia stimulates cerebral centres which send down impulses causing inhibition of the knee jerk.

Effects of oxygen lack

(1) *Inhalation of 6–8 p.c. oxygen mixtures in animals with vaso-sensory nerves intact.* There may be an increase in quadriceps tone which is slight and variable in occurrence. There is always, however, a diminution in the amplitude of the knee jerk, but this is not very great. Winkler [1929] on the other hand, using the reflex contraction of the tibialis anticus, obtained a regular increase in the size of the response during inhalation of mixtures containing 10 p.c. oxygen or less. We are unable to account for this discrepancy in the results.

(2) *Inhalation of nitrogen containing 2–3 p.c. oxygen.* In our experience two types of response may be obtained:

(*a*) There is a short-lived initial depression followed by a recovery of variable extent after which a final depression develops. The initial depression is doubtless due to progressive anoxia. The second phase can be attributed to the transient stimulating action of very grave anoxia. The final depression is the second effect of such grave anoxia. On air, recovery gradually occurs (Fig. 7 A).

(*b*) There may be a marked initial increase in quadriceps tone accompanied by a progressive and rapid decrease and final abolition of the knee jerk. When air is readmitted, recovery sets in with loss of excessive tone and increase in the amplitude of the jerk (Fig. 7 B).

In experiments on two animals with the spinal cord divided in the mid-thoracic region the inhalation of nitrogen produced results similar to those recorded in (2 (*b*)).

(3) *Inhalation of 6–8 p.c. oxygen mixtures in animals with vagi cut and sinuses denervated.* Under these conditions, as shown by Wright [1936], the resting pulmonary ventilation is reduced and the breathing is quickly depressed by the inhalation of oxygen-poor mixtures. The oxygen content of the blood rapidly declines during such inhalations, so that with 8 p.c.

Fig. 7. A. Cat. Chloralose (0·08 g. per kg.). Records from above downwards are knee jerk, blood pressure, time in min. and signal line. Inhale nitrogen. B. Another animal showing the second type of response to the inhalation of nitrogen.

oxygen in the inspired air a degree of oxygen lack is induced which is little less severe or acute than that resulting from the inhalation of nitrogen in the intact animal. The effects observed are similar to those described under (2 (*a*)) above. Comparing again the effects on the knee jerk and on respiration it should be stressed that under these conditions no stimulation of the denervated respiratory centre takes place [e.g. Selladurai & Wright, 1932; Wright, 1936].

Effects of alteration in the carbon dioxide pressure in the blood

This subject has been extensively studied by King *et al.* [1932] and in general the results obtained in our small number of experiments are in agreement with their findings. Vigorous over-ventilation leads to some increase in the knee jerk and this is accompanied by the twitching characteristic of tetany [see Lennox & Cobb, 1928]. According to McDowall [1930], however, in decerebrate animals over-ventilation diminishes muscle tone. The inhalation of 7 p.c. CO_2 mixtures causes a slight depression of the knee jerk. In our hands, the changes produced by quite large variations in the CO_2 pressure in the blood were of a relatively insignificant character. It may be concluded that CO_2 accumulation plays an unimportant part in the responses described above following complete central anæmia.

Asphyxia produced by obstruction of the tracheal cannula leads to a rapid marked decrease of the knee jerk. This observation again emphasizes the greater importance of oxygen lack as a depressant factor. Sherrington [1910] and Mathison [1910] showed that asphyxia facilitates the scratch reflex in spinal animals, but it must be remembered that our experiments differed in two important respects—the spinal cord was intact and the animals were under the influence of an anæsthetic. Kaya & Starling [1909] have previously emphasized the differences in the response of animals with spinal cord intact and divided to various chemical agencies.

Action of cyanide

With doses of the order of 1 mg., the blood pressure is lowered, breathing is stimulated, and the knee jerk is depressed but only for a short period. There is then a smooth recovery (Fig. 8). With bigger doses, e.g. 2 mg., the effects on the knee jerk closely resemble those obtained with nitrogen, complete anæmia or inhalation of oxygen poor mixtures in the denervated preparation. There is (i) an initial depression, (ii) a temporary recovery and then (iii) an abolition of the reflex for varying periods of time (Fig. 8). During the second phase there may be generalized convulsive movements, increase of quadriceps tone, and the tap on the patellar tendon may elicit the crossed extensor reflex in the opposite hindlimb or in the forelimbs. When the knee jerk disappears these more generalized reflex activities also disappear. The crossed extensor reflex may, however, reappear before the knee jerk does. Different reflexes may thus be affected in different chronological order.

Either of the first two phases ((i) and (ii)) may be absent, i.e. there may be an initial stimulation followed by depression or there may be depression from the commencement.

Fig. 8. Cat. Chloralose (0·08 g. per kg.). Weight 5·5 kg. Records from above downwards are oxygen consumption with Krogh's spirometer, knee jerk, blood pressure and time in min. Inject at *A*, 1 and at *B*, 2 mg. sodium cyanide.

Examination of the oxygen consumption curve (Fig. 8) shows that the secondary stimulation (phase (ii)) of the knee jerk coincides with the period of depressed metabolism and can probably be attributed to the acute anoxia which is thus produced. It must be stressed, however, that the final disappearance of the knee jerk usually takes place when

the metabolism has returned to normal or has even risen above normal. These results can be interpreted in the light of the observations recorded above on the effects of acute anæmia. The nerve cells are originally stimulated by anoxia and then depressed, and once such paralysis or "narcosis", as Mathison [1910] calls it, has set in, restoration of the metabolism or of the oxygen supply cannot restore activity until after the passage of a considerable period of time. In some of our experiments, recovery following the injection of cyanide has not taken place until after 20 min. Our results differ from those of Glazer [1929], who only observed an increase in reflex responses following the injection of cyanide.

In animals with the spinal cord divided in the mid-thoracic region, cyanide produces effects on the knee jerk similar to those described in the intact animal. These results are, therefore, due to the direct action of the drug on the spinal centres.

We have compared the effects of cyanide on respiration and on the knee jerk. In the innervated animal cyanide produces an initial stimulation of respiration which is followed by a prolonged secondary stimulation which long outlasts the period of reduced metabolism (e.g. Fig. 8) [Wright, 1935]. The period of initial respiratory stimulation may coincide with phase (ii) of the knee jerk effect, but the secondary increase in breathing usually overlaps the period of abolition of the knee jerk. In the denervated animal the action of cyanide on the knee jerk is unaltered, but instead of an initial stimulation of respiration there is a pure depression of the breathing (Fig. 9). Cyanide, therefore, affects the respiratory and spinal centres quite differently.

Fig. 9. Cat. Chloralose (0·07 g. per kg.). Weight 2·2 kg. Sinuses denervated and vagi cut. Records from above downwards are oxygen consumption with Krogh's spirometer, knee jerk, blood pressure and time in min. Inject at *A* 0·25, at *B* 0·5, at *C* 1 mg. sodium cyanide.

Discussion

The evidence presented shows that stimulation of the central end of the vagus reflexly depresses the knee jerk by a direct action on the central nervous system, and that the effects obtained are not dependent on the associated circulatory or respiratory changes. These results demonstrate that irradiation may extend for long distances in the central nervous system, in this instance from the vagus nucleus in the medulla to the lumbar region of the spinal cord. As has been indicated, the features characteristic of pure somatic reflexes such as recruitment and after-discharge [Sherrington], and which are also found [Wright, 1928] in pure autonomic reflexes, e.g. vascular reflexes of vagal origin, also apply to the mixed autonomic-somatic reflex studied in our experiments. Schweitzer [1936] has demonstrated the widespread nature of the irradiation which is set up by impulses in the vaso-sensory and other autonomic nerves and which affects many visceral functions. As the results obtained by means of central vagus stimulation on skeletal muscle reflexes agree generally with those produced by raising the pressure in the carotid sinus, it is possible that the afferents chiefly involved in our experiments are those arising in the aortic arch.

As has already been pointed out we have no evidence to support the view proposed by Dikshit that acetylcholine is a transmitter in these reflex reactions.

Our results with complete anæmia of the central nervous system, anoxia and changes in the carbon dioxide pressure in the blood are in general agreement with those obtained by the majority of previous observers.

We have stressed that the cells of the respiratory centre and those of the lumbar spinal cord react frequently quite differently to various alterations in their physico-chemical environment. This difference in the responses of the two centres is particularly well shown in our results on the effects of cyanide on the breathing and the knee jerk in animals with the vaso-sensory nerves divided.

Summary and conclusions

1. The effects on the knee jerk of stimulation of the central end of the vagus, lowering the blood pressure by various methods, acute complete anæmia of the central nervous system, anoxia, alterations in the carbon dioxide pressure in the blood and the intravenous injections of various doses of histamine and cyanide, have been investigated in the cat under chloralose anæsthesia.

2. Stimulation of the central end of the vagus produces a temporary depression or abolition of the knee jerk, frequently associated with a decrease in quadriceps tone. When afferent vagal stimulation is discontinued recovery sets in gradually.

3. The inhibitory effect of central vagal stimulation is not due to alterations in the condition of the quadriceps muscle itself; this has been shown by comparison of the knee jerk with the response of the quadriceps to stimulation of the femoral nerve during a period of central vagus stimulation.

4. The influence of central vagal stimulation on the knee jerk is independent of the simultaneous circulatory or respiratory changes. The impulses set up by stimulation of the central end of the vagus may be conducted in afferent fibres which arise in the aortic arch.

5. The results obtained demonstrate that afferent impulses in autonomic nerves may irradiate widely in the central nervous system and affect lumbar somatic centres. No evidence was obtained that acetylcholine acts as a transmitter in these reflex reactions.

6. Histamine has an inhibitory effect in the knee jerk owing in part to a central depressant action which is independent of the associated fall of blood pressure.

7. The effect on the knee jerk of obstruction of the abdominal aorta and inferior vena cava for periods up to 15 min. is negligible. Acute complete anæmia of the central nervous system produced by clamping the aortic arch depresses and abolishes the knee jerk after an initial phase of stimulation.

8. Anoxia produces variable effects on the knee jerk depending mainly on its severity and acuteness. The initial effect is inconstant, but commonly there is some degree of initial stimulation followed by secondary depression. Asphyxia leads to a rapid decrease of the knee jerk. The changes produced by gross alterations in the CO_2 tension in the blood are relatively insignificant.

9. The effects of cyanide on the knee jerk are similar to those produced by acute anoxia, and are probably produced by that means.

10. The effects of acute cerebral anæmia, severe anoxia and of cyanide on the knee jerk are not modified by transection of the spinal cord. The results described are, therefore, due to the direct action of these procedures on the lower spinal centres.

11. The cells of the denervated respiratory centre and those of the lumbar spinal cord react frequently quite differently to various alterations in their physico-chemical environment.

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612.833.94:577.174.5

THE ACTION OF ADRENALINE ON THE KNEE JERK

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(*Received* 10 *September* 1936)

In a previous paper [Schweitzer & Wright, 1937] we described the effects of central vagus stimulation on the knee jerk in cats under chloralose anæsthesia. The usual result obtained was an inhibition of the reflex, and it was suggested that the aortic fibres in the vagus nerve were responsible for the changes observed. Several investigators [Koch, 1931, 1932; Schweitzer, 1934, 1935] have demonstrated that the afferent impulses set up by raising the blood pressure in the carotid sinuses are also capable of inhibiting reflex activities of skeletal muscle. It was, therefore, thought of interest to study the effects of pressor doses of adrenaline on the knee jerk, as it is well known that the rise of blood pressure produced by this substance stimulates the vaso-sensory terminals and reflexly inhibits a number of functions such as the heart rate, respiration and the tonic discharge of the vaso-motor centre.

The only reference that we have been able to find to the action of adrenaline on muscular reflexes is a short note by Spychala [1932], who while investigating the Orbeli phenomenon compared the effects on the knee jerk of the injection of adrenaline with those of peripheral sympathetic nerve stimulation. He found that adrenaline usually produced some increase in the reflex response but occasionally noted an inhibition which occurred at the height of the rise of blood pressure. He seems to relate his results to the peripheral action of the sympathetic on skeletal muscle.

A preliminary report of our studies on the action of adrenaline on the knee jerk has already been made [Schweitzer & Wright, 1936].

Methods

The methods employed for eliciting and recording the knee jerk and for peripheral stimulation of the motor nerve to the quadriceps muscle were the same as those described in an earlier paper [Schweitzer & Wright, 1937]. As previously, the response of the quadriceps was studied both when free-weighted and after-loaded.

Results

Intravenous injection into cats under chloralose anæsthesia (0·065–0·08 g. per kg. body weight) of adrenaline in doses of 0·2–0·4 mg. (0·05–0·15 mg. per kg.) produces various effects on the knee jerk.

(1) The usual result obtained is a depression or abolition of the jerk often accompanied by some decrease in muscle tone (Fig. 1). The inhibition sets in after a latent period which is usually not shorter than 30 sec., but is often considerably longer and may extend to 3 or 4 min. The inhibition then progressively becomes more marked and reaches its maximum after 4–14 min. In some experiments the reflex may then remain in abeyance for as long as 10 min. Finally, recovery sets in gradually and is usually complete after 10–90 min.; not infrequently, however, complete recovery does not occur. The return of muscle tone usually precedes the full recovery of the knee jerk to its normal level.

Using the technique described a decrease in tone can, of course, be observed only when the limb is allowed to hang freely; it then shows itself by increased flexion at the knee joint. The degree of passive stretch of the quadriceps remains, however, unchanged, so that patellar stimulation presumably stretches the muscle to the same degree as previously. When the experiment is carried out with the leg supported, a decrease in tone leads to slackening of the quadriceps and to a corresponding diminution in the effectiveness of the stretching force exercised by the hammer blow on the tendon. A decrease in the extent of the knee jerk under such conditions must be due in part to the associated diminution in tone. Both experimental procedures do, however, give a measure of the extent to which adrenaline modifies proprioceptive reflexes.

(2) Very commonly there is, preceding the inhibition, an initial increase in the size of the response, the peak being gradually attained after about 3 min. The initial increase in tension may be considerable and amount to 40–100 p.c. of the initial value. The inhibition then gradually sets in as already described. In occasional experiments the initial stimu-

lation is the outstanding feature of the response and the secondary inhibition is slight or absent.

(3) Rarely a "triple reaction" may be obtained which consists essentially of reaction (2) preceded by a short initial depression. Fig. 2 illustrates one of these results and shows an initial rapidly developing short-lived depression, followed by a secondary stimulation and then by a final inhibition which in its turn is followed by the usual slow recovery.

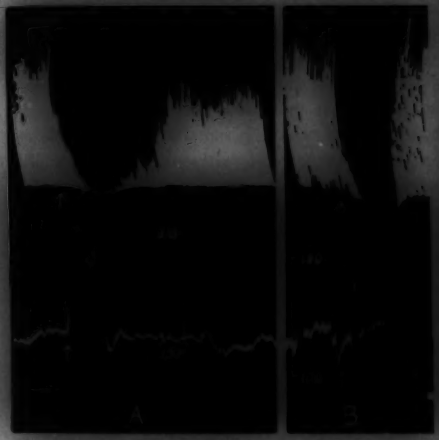

Fig. 1.

Fig. 2.

Fig. 1. Cat. Chloralose anæsthesia. Records from above downwards: knee jerk (free-weighted), blood pressure, time in 30 sec. A. Inject 0·4 mg. adrenaline. B. Carotid sinuses denervated and vagi cut. Inject 0·4 mg. adrenaline.

Fig. 2. Cat. Chloralose anæsthesia. Records from above downwards: knee jerk (after-loaded), quadriceps of other side stimulated through its motor nerve, blood pressure, time in 30 sec. Inject 0·4 mg. adrenaline.

(4) It can readily be shown that adrenaline also depresses other muscular activities. If the anæsthetic is light and the animal shows spontaneous movements these may be found to disappear after the injection of adrenaline at the same time as the knee jerk declines, and to return as the knee jerk recovers.

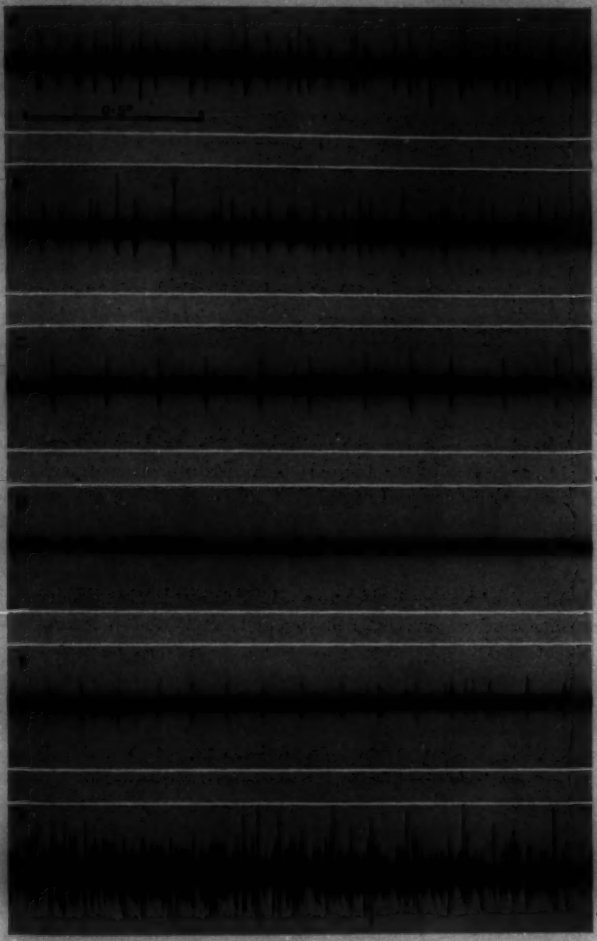

Fig. 3. Cat. Decerebrate. Records of action currents in quadriceps muscle (Adrian and Bronk concentric needle electrode and cathode ray oscillograph). A. Prior to injection. B. 22 sec. after injection of 0·2 mg. adrenaline. C. After 63 sec. D. After 90 sec. E. After 24 min. F. After 38 min. (From an experiment by W. Floyd and C. A. Keele.)

(5) Changes in muscle tone produced by adrenaline have been more fully studied by Floyd & Keele [1936] in the decerebrate animal by means of an Adrian and Bronk concentric needle electrode which is introduced into the quadriceps muscle and connected with an amplifier and cathode-ray oscillograph unit. In general they found that the intravenous injection of adrenaline produced: (i) a brief cessation of activity lasting for a few seconds which occurs about 15 sec. after the injection; (ii) a return of the original electrical activity which in some cases is enhanced; (iii) a decline in electrical activity which finally ceases completely at from 45 to 90 sec. after the injection; (iv) a slight gradual recovery to the original level at 3 to 40 min. after the injection; sometimes activity returns and ceases alternately before attaining the pre-injection level. In other cases the electrical activity was subsequently enhanced.

Fig. 3 is a typical record to show the results obtained by them. In this experiment considerable electrical activity was present in the quadriceps (Fig. 3 A), and was found to be steadily maintained for a period of 1 hour prior to the injection. 22 sec. after the injection of 0·2 mg. of adrenaline (Fig. 3 B) electrical activity was definitely depressed, and after 63 sec. (Fig. 3 C) only two motor units are observed to be discharging slowly. Electrical activity ceased completely at 90 sec. (Fig. 3 D) and remained in abeyance for about 20 min. Activity began to return at 24 min. (Fig. 3 E) and at 38 min. was more violent than prior to the injection (Fig. 3 F).

In the decerebrate preparation the decrease in tone produced by adrenaline is general in its distribution and can be readily demonstrated by palpation.

Mechanism of adrenaline action

The following possible modes of action of adrenaline on the knee jerk were considered and experimentally investigated.

(1) Presence of the usual chloretone preservative in the adrenaline solution.

(2) Peripheral action on the quadriceps.

(3) Coincident changes in respiration.

(4) Reflex action owing to the rise of blood pressure stimulating the vaso-sensory nerves in the sino-aortic areas or elsewhere.

(5) Liberation of a chemical transmitter, especially acetylcholine.

(6) Constriction of the blood vessels diminishing the blood flow through the central nervous system and directly or indirectly modifying the responses of the lumbar spinal centres.

(7) Rise of blood pressure acting directly on the central nervous system.

(8) Adrenaline acting directly on the neurones of the central nervous system.

(1) *Action of preservative*

The adrenaline solution employed contains 0·5 p.c. chloretone. The repeated injection of 1 c.c. of 0·5 p.c. chloretone has no effect whatever on the knee jerk. This is a larger amount than is present in the doses of adrenaline employed. The preservative, therefore, plays no part in producing the adrenaline reaction.

(2) *Peripheral action of adrenaline*

Previous workers have shown [Gruber, 1922, 1924] that in the mammal adrenaline may increase the response of skeletal muscle to peripheral motor nerve stimulation. It is, therefore, exceedingly improbable that the adrenaline inhibition of the knee jerk which has just been described can be attributed to a peripheral action of the drug on the muscle. It is possible, however, that the initial increase in the knee jerk often observed might be produced in this way. We carried out a number of experiments in which we compared simultaneously the effects of adrenaline on the knee jerk on the right side and on the peripherally stimulated quadriceps muscle on the left side (cf. Fig. 2). In none of our experiments was there any modification of the response of the peripherally stimulated muscle (apart from that attributable to fatigue) no matter what the type of alteration in the knee jerk happened to be. It is clear, therefore, that the peripheral action of adrenaline can be excluded as a significant factor in our results.

(3) *Changes in respiration*

No relationship can be established between the effects on the knee jerk and those on respiration. When the sinuses and vagi are intact it is found that the adrenaline apnœa passes off and breathing returns to normal before the inhibition of the knee jerk begins to set in. The breathing may be in the phase of secondary stimulation due to the direct action of adrenaline on the respiratory centre [Wright, 1930*b*] when the inhibition of the knee jerk is at its maximum.

(4) *Reflex action through the vaso-sensory nerves*

The changes in the knee jerk are likewise not closely related to those in blood pressure. Characteristically they lag behind the alterations in the blood pressure. Thus the rise of blood pressure attains its maximum

height before the inhibition of the knee jerk begins. Again the blood pressure commences to fall when the inhibition of the reflex is deepening. Frequently the blood pressure has returned to its pre-injection level before any indication of recovery of the knee jerk is noted.

The action of adrenaline was studied in animals with vagi cut and carotid sinuses denervated ("denervated" animals). It was found in such preparations that adrenaline still produces the usual characteristic inhibition of the knee jerk (Fig. 1 B). Floyd and Keele, who examined the question for us, similarly found in electrical studies on the quadriceps muscle of the decerebrate cat that the response to adrenaline was

Fig. 4. Cat. Chloralose anaesthesia. Spinal cord divided in mid-thoracic region. Records from above downwards: knee jerk (after-loaded), blood pressure, time in 30 sec. Inject 0·4 mg. adrenaline.

unaffected by vaso-sensory denervation. The inhibition of the knee jerk is also obtainable after acute division of the spinal cord in the mid-thoracic region (Fig. 4). It can be concluded, therefore, that the effects of adrenaline are not essentially dependent on afferent impulses in the sino-aortic nerves. The experiments on the spinal animal also exclude the possibility that the afferent impulses which may be set up by blood pressure changes in the pulmonary circuit [Schwiegk, 1935; Schweitzer, 1936] are necessary for the responses obtained.

The role of other vaso-sensory nerves still remains to be considered. Gammon & Bronk [1935] showed that afferent impulses initiated by vascular distension may be set up in sensory nerve endings in the splanchnic area but offered no satisfactory proof that these were of functional significance. Heymans *et al.* [1936], however, produced

definite evidence that impulses set up by changes in mesenteric blood pressure can reflexly modify general vascular tone. They obtained similar results in the spinal animal but not following destruction of the medulla. They concluded that the medulla is an integral part of the reflex arc employed. Our experiments on the spinal animal demonstrate that vaso-sensory impulses which pass through the medulla, no matter what their origin, are not indispensable for the adrenaline effect on the knee jerk. It is possible, however, that the afferent impulses set up by adrenaline may pass directly from the splanchnic area to the lumbar spinal centres and produce inhibition there. To test this hypothesis experiments were performed in which animals were "denervated" and the splanchnic nerves which transmit the afferent impulses from the mesenteric vascular region were also divided. In such preparations adrenaline still produces its characteristic effects on the knee jerk. It may be concluded, therefore, that vaso-sensory impulses, no matter what their seat of origin, are not essential for the production of the adrenaline response.

Another point that may be mentioned which opposes the reflex hypothesis of the adrenaline action is that following central vagus stimulation the reflex inhibition of the knee jerk sets in almost immediately, while with adrenaline there is always a delay of at least 30 sec. and frequently of much longer duration.

It is useful to contrast with the foregoing results the analysis of the inhibition of the vaso-motor centre which is produced by adrenaline. Adrian *et al.* [1932] found that injection of 0·5–1 mg. of adrenaline intravenously in the rabbit arrested the efferent discharge in sympathetic fibres for 5 to 10 min. owing to an inhibition of the vaso-motor centre. This inhibitory action was almost completely abolished following section of the cardiac depressor nerves and tying the carotid arteries above and below the carotid sinus. This procedure does not, of course, completely denervate the vaso-sensory zones and it may be concluded, therefore, that the inhibitory action of adrenaline on the vaso-motor centre is wholly reflex in character.

(5) *Possible liberation of a chemical transmitter by adrenaline*

Feldberg & Schriever [1936] found that adrenaline in the eserinized animal causes the appearance of acetyl-choline in the cerebro-spinal fluid. We have shown [Schweitzer & Wright, 1936] that acetylcholine can markedly inhibit the knee jerk and it was, therefore, necessary to consider whether the action of adrenaline on the knee jerk was in part due to the mediation of this transmitter. A number of experiments were

performed in which animals were fully atropinized and then given an injection of 1 mg. eserine per kg. body weight. Under these conditions adrenaline still produces its characteristic inhibitory effects on the knee jerk but its action is not potentiated. There is, therefore, no reason to suppose that acetylcholine is concerned as an intermediary in the adrenaline reaction.

(6) *and* (7) *Role of vaso-constriction and direct action of rise of blood pressure*

The next possibilities that have to be considered are whether the rise of blood pressure or the vaso-constriction produced by adrenaline are the responsible factors. The following observations bear on the elucidation of this question:

(i) It has already been mentioned that there is no direct time relationship between the changes in the blood pressure and in the knee jerk.

(ii) In some experiments, where the rise of blood pressure produced by adrenaline has been small and very transient, a marked inhibition of the knee jerk has yet been obtained (Fig. 1 B).

(iii) In animals with vagi previously divided, occlusion of both common carotid arteries produces a marked sudden rise of blood pressure which equals in extent and acuteness that produced by an appropriate injection of adrenaline. In the experiment illustrated by Fig. 5, clipping the carotid arteries caused the blood pressure to rise from 190 to 260 mm., but the knee jerk showed only a negligible modification. A subsequent injection of 0·4 mg. adrenaline which raised the blood pressure equally rapidly to 260 mm. produced, after an initial stimulation, the characteristic and long-lasting abolition of the knee jerk, followed by a slow recovery. This experiment shows particularly well how the blood pressure may fall to below its pre-injection level long before the inhibition of the knee jerk becomes maximal. Such experiments exclude the possibility of the rise of blood pressure *per se* being responsible for the results.

In one experiment, however, carotid occlusion did produce a marked decrease in the knee jerk. The same result was still obtained after the suprarenal glands were removed to exclude the possibility of adrenaline being discharged into the circulation. It has, however, been shown previously [Schweitzer & Wright, 1937] that cerebral anæmia depresses the knee jerk, probably by causing the medullary centres to discharge inhibitory impulses to the lumbar cord [Montgomery & Luckhardt, 1930]. The exceptional result with carotid occlusion can probably be explained on such lines.

Fig. 5. Cat. Light chloralose anæsthesia (0·065 g. per kg.). Both vagi cut. Left carotid sinus denervated. Suprarenals excluded. Records from above downwards: knee jerk (after-loaded), blood pressure, time in min. At first arrow: clip right carotid artery. At second arrow: remove clip. At third arrow: inject 0·4 mg. adrenaline. The irregularities in the knee jerk are mainly due to the light anæsthesia.

Fig. 6. Cat. Chloralose anæsthesia. Records from above downwards: knee jerk (after-loaded), blood pressure, time in min. Inject successively 10 mg. tyramine, 0·4 mg. adrenaline.

(iv) The effects of other pressor drugs were compared with those of adrenaline. Fig. 6 shows that the injection of 10 mg. tyramine may raise the blood pressure to 250 mm. without affecting the knee jerk at all, while the subsequent injection of adrenaline which produced an equal rise of blood pressure caused the characteristic inhibition. When very large doses of tyramine are employed, e.g. about 100 mg., they produce as a rule a gradual but lasting depression of the knee jerk which is sometimes completely abolished. Respiration may then fail and death ensues. These results are doubtless due to a direct poisonous action of large doses of the drug.

Similar negative results have been obtained with large pressor doses of pituitrin (5 units).

(v) In some experiments, when adrenaline is injected after the carotid arteries have been clipped, it may produce no further elevation of the blood pressure but still gives rise to the typical inhibition of the knee jerk.

In these experiments the important fact to consider is not the degree of general vaso-constriction but the extent to which it affects the blood vessels of the central nervous system. We are not aware of any work on the changes produced by adrenaline in the spinal blood vessels which are those that particularly concern us, but for the moment it would presumably be safe to suppose that they are of the same nature as those taking place in the cerebral blood vessels. Forbes & Wolff [1928] found in six experiments that when adrenaline was injected intravenously in large pressor doses pure constriction of the cerebral vessels did not occur; in four cases constriction took place following an initial dilatation, and in two a pure dilatation occurred. Fig. 14 of their paper shows that the maximal decrease in vessel diameter was 33 p.c. and that the diameter of the pial vessels returned to normal at about the same rate as the blood pressure. In our experiments it has been clear that there is a long time lag between the restoration of the blood pressure and the recovery of the knee jerk. Furthermore, our previously reported observations on the effects of anæmia and anoxia showed clearly that to produce a complete abolition of the knee jerk such as occurs so frequently with adrenaline, it is necessary for the oxygen supply of the central nervous system to be completely cut off for some minutes. It is obvious from the account of Forbes & Wolff that such a state of affairs does not occur following injections of adrenaline.

These experiments and theoretical considerations taken together demonstrate that neither the rise of blood pressure nor the vaso-constriction set up by adrenaline plays any significant part in producing its effects on the knee jerk.

(8) *Direct action of adrenaline on the central nervous system*

The last possibility to be considered is that adrenaline acts directly on the components of the central nervous system. This view is supported by the following observation. It is well known that an increase in the amount of adrenaline injected, once a certain dose has been reached, does not produce a corresponding increase in the rise of blood pressure. Fig. 7 shows an experiment in which the successive injections of 0·05, 0·2, 0·4 mg. adrenaline raised the blood pressure to approximately equal levels, i.e. to about 200 mm. The effect on the knee jerk, however, was proportional to the dose of adrenaline employed: it was negligible with 0·05 mg.; there was a slight depression following the initial stimulation with 0·2 mg.; with 0·4 mg. there was complete inhibition following the initial stimulation, followed by a very gradual process of recovery.

Action of ergotoxin. Ergotoxin paralyses certain of the motor actions of adrenaline through its effects on peripheral nerve endings [Dale, 1906]. It was, therefore, thought of interest to see whether it had any influence on the central actions of adrenaline which have been described in this paper. The problem is complicated by the fact that ergotoxin itself directly modifies the knee jerk in a variable and often marked manner. It is commonly found that the dose of ergotoxin which is necessary to delay or reverse the pressor action of adrenaline, itself depresses or even abolishes the knee jerk permanently. Different preparations of the drug were found to differ among themselves in respect of this action. There is evidence too from other sources [Wright, 1930*a*; Heymans *et al.* 1930] that ergotoxin depresses the medullary centres such as the vaso-motor and the respiratory. In a few experiments, however, we were successful in reversing the vascular action of adrenaline by means of ergotoxin without adversely affecting the knee jerk to a serious extent. In such cases the injection of 0·4–1 mg. adrenaline failed to produce the characteristic effects on the knee jerk. We do not wish to stress these results unduly but they suggest the possibility that under some circumstances ergotoxin may antagonize the central as well as certain of the peripheral actions of adrenaline.

Action of drugs which potentiate the peripheral action of adrenaline. According to Bacq [1936] cocaine, pyrogallol and thyroxine sensitize certain organs to sympathetic stimulation and to the injection of adrenaline. We have carried out experiments with all these substances but in our hands they produced no modification of the adrenaline action on the knee jerk.

Fig. 7. Cat. Chloralose anæsthesia (0·07 g. per kg.). Both vagi cut. Records from above downwards: knee jerk (after-loaded), blood pressure, time in min., signal line. Inject successively 0·03, 0·2, 0·4 mg. adrenaline.

Discussion

The facts reported demonstrate that adrenaline acts directly on the elements of the central nervous system and more especially on the lumbar spinal somatic centres. The effects of adrenaline on the knee jerk cannot be reproduced by other procedures which cause a similar degree of vaso-constriction or an equal rise of blood pressure. It is certain, therefore, that the vascular changes produced by adrenaline acting either directly or reflexly are not the main factors responsible for producing the alterations in the knee jerk. This conclusion is strengthened by the fact that the knee jerk changes are not significantly modified when adrenaline is injected following denervation of the sino-aortic areas and exclusion of the mesenteric and pulmonary vascular afferents. Even when the more refined method is employed of studying the action currents due to muscle tone in the quadriceps in the decerebrate animal, it cannot be shown that vaso-sensory denervation alters the adrenaline response. These results surprised us in view of the evidence already quoted that afferent impulses from the carotid sinuses and experimental stimulation of the central end of the vagus may produce a considerable degree of inhibition of muscle tone and skeletal reflex activity. Our observations, however, do not absolutely exclude the possibility that vaso-sensory impulses play a minor part during the adrenaline reaction in the intact animal.

We have come all the more unwillingly to these conclusions because the research arose from the theory that injection of adrenaline would inhibit skeletal muscle activities exclusively reflexly from the vaso-sensory zones.

Although the regular response to adrenaline is inhibition, we must admit that initial stimulation occurs with sufficient frequency to merit consideration as a normal component of the reaction. We have not been able to discover the factors which determine the type of response that is to be obtained. In our small number of experiments on the spinal animal initial stimulation has not occurred and the possibility exists that adrenaline may act differently on the spinal and higher centres.

Emphasis has been laid on the remarkably prolonged nature of the adrenaline action on the central nervous system. As we have mentioned, in some experiments 90 min. may elapse before the pre-injection condition is restored. In this connexion it is of interest to draw attention to the experiments of Palme [1936], who found that painting the exterior of the carotid sinus with a solution of 1/1000 adrenaline produced a reflex fall of blood pressure which lasted for 40–60 min. This

is another piece of evidence that adrenaline may act on nervous elements other than the post-ganglionic terminals of the sympathetic and that its action in such cases may be very prolonged. It has been shown that the destruction of adrenaline in the body is effected by specific enzymes, but it does not yet seem to have been determined whether these substances are found in nervous tissues or in what concentrations (Blaschko). Such information might throw considerable light on the prolonged duration of the central action of adrenaline.

It is premature to speculate at the moment about the exact way in which adrenaline modifies the behaviour of somatic centres. It would be of interest to know whether in appropriate concentrations it can modify the oxidative or other metabolic processes of slices of spinal cord *in vitro*.

We do not wish at present to suggest that our experiments have other than a purely pharmacological significance, as the doses of adrenaline that we have to employ to produce our results are far greater than are ever supposed to be secreted in the body; but if one were tempted to consider the results from the physiological standpoint, it is obvious that they are contrary to the generally accepted idea of the emergency function of the adrenal gland, unless we suppose that "passive resistance" is the most appropriate response to certain situations of danger. Nor do we wish to suggest that adrenaline is concerned as a transmitter in skeletal muscle reflexes, though it may possibly modify the formation or removal of any transmitter that may be concerned, or to use Sherrington's terminology, it may influence the central excitatory or inhibitory states.

Summary

1. The action of adrenaline on the knee jerk has been studied in cats under chloralose anæsthesia. Intravenous injection of 0·2–0·4 mg. adrenaline usually produces a diminution or abolition of the knee jerk accompanied by a decrease in muscle tone. Recovery sets in gradually and is complete in 10–90 min. Initial stimulation often precedes the inhibition or there may be a "triple response" consisting successively of depression, stimulation and depression. Floyd & Keele have demonstrated that adrenaline abolishes the action currents due to muscle tone in the quadriceps of the decerebrate cat.

2. The effects described are not due to the chloretone preservative nor to the peripheral action of adrenaline on the quadriceps muscle itself.

3. The adrenaline inhibition is still obtained following denervation of the sino-aortic pulmonary and mesenteric vascular areas or division of the spinal cord in the mid-thoracic region.

4. The adrenaline effects are not reproduced when a similar degree of vaso-constriction and rise of blood pressure is brought about in other ways, such as by occlusion of the carotid arteries (in animals with vagi cut), or by injection of tyramine or pituitrin.

5. Increasing doses of adrenaline, though causing equal rises of blood pressure, produce a progressively greater degree of inhibition of the knee jerk.

6. The action of adrenaline is not potentiated by eserine, cocaine, pyrogallol or thyroxine. It is sometimes antagonized by ergotoxin.

7. It is concluded that adrenaline acts on the knee jerk by a direct effect on the elements of the nervous system. The exact nature of this central action is not known.

8. The significance of these results is discussed.

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612.731.63:612.014.462.7

THE CHANGES IN VISCOSITY OF AN UNSTRIATED MUSCLE (*MYTILUS EDULIS*) DURING AND AFTER STIMULATION WITH ALTERNATING, INTERRUPTED AND UNINTERRUPTED DIRECT CURRENTS

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(*Received* 18 *September* 1936)

Muscle viscosity influences the response to a stimulus in two ways, by opposing rapid shortening or development of tension, and by retarding relaxation. The importance of the former is illustrated by the large reduction in viscosity undergone by the retractor penis of the dog when stimulated with adrenaline [Winton, 1930]: the muscle is changed from a condition in which it is capable of resisting external forces passively and economically, to one in which it can pull relatively powerfully and quickly. The importance of a high viscosity in slowing the relaxation, on the other hand, is due to the consequence that a fused contraction is obtained in plain muscle even with a low frequency of stimulation; the increase of oxygen consumption required to maintain a tetanus might, therefore, be too low to be estimated. Bayliss [1928] showed that this consideration met the objections to the tetanus theory of tonus which had been based on failure to detect an increase of oxygen consumption during tonic contraction. The tetanus theory encounters, however, another difficulty, in that a frequency of stimulus high enough to produce a fused response ordinarily results in the rapid onset of fatigue in plain muscle, whereas normal tonic contraction appears to be unattended by fatigue. A stimulus applied once a minute, for example, produces fatigue with relatively little fusion both in the retractor penis of the dog [Winton, 1926, Fig. 1] and in the anterior retractor of *Mytilus* (Fig. 1 B below). Stimulation at a frequency high enough to produce a fused contraction induces fatigue even sooner. If, however, it were found that not all kinds of stimuli reduce the viscosity of plain muscle, but that

suitable stimuli may even increase the viscosity, the least frequency of stimulation needed to produce a fused response would be correspondingly lower; consequently the muscle would have time to recover from the ill effects of one stimulus before the next appeared, and fatigue would not develop. It happens that in the anterior retractor of *Mytilus* a direct current stimulus increases the viscosity and an interrupted or alternating current stimulus decreases the viscosity, the changes in viscosity greatly outlasting the duration of the stimulus [Winton, 1934]; this muscle has, therefore, been chosen for studying the changes of viscosity in virtue of which a tetanus theory of tonus may be tenable in plain muscle. In view of the sensitivity of plain muscle to chemical stimulation, it is unlikely that all examples of tonic contraction are due to discontinuous stimulation; the "restoring force" previously described [1930] as present in the isolated retractor penis, and also operative in the isolated retractor of *Mytilus* (Fig. 5 below), is clearly a different affair. The tetanus theory of tonus is particularly applicable to those instances which used to be ascribed to a "catch mechanism", in which nervous activity, possibly of the nature of a stretch reflex, is apparently involved. Since, as far as we know, nervous activity is discontinuous, the problem of how plain muscle yields a tonic response to discontinuous stimulation becomes an important one.

The exact meaning of the term viscosity in connexion with plain muscle was discussed in a previous communication [Winton, 1930]. The viscous-elastic forces with which a retractor penis resists stretch were shown to be capable of representation by a triple component mechanical model, consisting of pure elastic, viscous-elastic, and pure viscous elements, under the influence of a small restoring force which induced slow but ultimately full shortening of the muscle in the absence of an external stretching force large enough to overcome it. Stretch curves indicate that the triple model designed for the dog's retractor penis represents adequately, though not quite so satisfactorily, the mechanical properties of the *Mytilus* retractor, the straight part of the curve being shorter in most of these muscles. The two muscles have in common that they consist of parallel longitudinally arranged unstriated fibres, with relatively little adventitious tissue, and that they are of about the same size and shape, but the individual fibres in the *Mytilus* retractor are very much longer than those in the dog's retractor penis.

Technique. As with other plain muscle preparations, small differences in technique may produce surprisingly large changes in the reactions of the muscle; the experimental results described below were obtained with

the isolated anterior retractor of the byssus of *Mytilus edulis*, each end of the muscle being tied with thread and arranged in the stimulation chamber as previously described [Winton, 1926]. The muscles were immersed in sea water, and stimulated by currents passing through it; deep sea water and water from the aquarium tanks of the Plymouth Marine Biological Laboratory did not produce obviously different results, but certain other changes in the solution and aerating with 5 p.c. CO_2 in O_2 instead of with air affected the muscle so considerably that some of the properties described below became masked. Muscles obtained from different batches of animals showed different initial viscosities and variations in the degree of change in tension and viscosity due to various forms of stimulation. This did not appear to be due to the fact that some animals were used as soon as they were brought in from the boat, others after some time in the Plymouth tanks, and others after keeping in a refrigerator in Cambridge for anything up to a fortnight packed in seaweed as sent from Plymouth. If, however, mussels are in bad condition after having been overcrowded in a tank of sea water, their muscles are usually exceptionally viscid and respond poorly to alternating current stimulation.

Good muscles survive under the conditions named for many hours, comparable developments of tension being obtained for 4–6 hours, and good though smaller responses can be obtained 24 hours or more after isolation.

If the responses of any given series are to be comparable with one another, stimulation should be applied not more frequently than once in every 5 min. to avoid fatigue and at intervals not longer than 10–15 min. to avoid the staircase phenomenon which is well marked. Summer temperature is unfavourable for the maintenance of consistent results for long periods, and the experiments here described were mainly conducted, in a bath thermostatically controlled, at temperatures between 8 and 10° C. This temperature is substantially lower than the optimum for tension development.

Polarization was presumably occurring at the silver silver-chloride electrodes during the passage of the relatively large stimulating current, much of which passed through the sea water. The effects described below are, however, unchanged when about ten times the usual stimulating voltage is applied through an appropriate resistance in series with the stimulating chamber. Electrode polarization, therefore, plays no substantial part in producing the characteristic effects on the muscle of direct and alternating currents. Changes of concentration of ions in the

sea water due to electrolysis were shown to have a negligible influence by comparing the consequences of stimulation when the sea water in the stimulating chamber was being rapidly replaced by fresh sea water, with those obtained when the liquid in the chamber was stationary. The alternating current employed in these experiments, in which it is not specified that a commutator was used, was derived from the 50 c.p.s. mains supply through a transformer and potential divider.

Fig. 1. Isotonic responses of an isolated *Mytilus* muscle stimulated with fluid electrodes. Sea water. 9·2° C. A. Three contractions due to stimulation with A.C. (50 c.p.s.) for 10 sec. involve much quicker relaxation than that due to stimulation with D.C. of the same duration. B. Fatigue curve due to stimulation with A.C. once a minute—a frequency too low to produce considerable fusion of responses. C. The response to stimulation with D.C. once a minute, of the same voltage and duration, showing summation and almost complete fusion.

The contrast in the effects of stimulation with A.C. *and* D.C. Fig. 1A shows isotonic responses to stimulation for 10 sec. with alternating currents (A.C.) and direct currents (D.C.). In the former the muscle has practically relaxed in a minute; in the latter the same degree of relaxation occupies over an hour. Records of isometric responses to the two

forms of stimulation look about the same as records of isotonic responses.

Consider the consequences of repetitive stimulation with the two different kinds of current. If A.C. stimuli of 10–15 sec. duration are applied at varying intervals, there is an optimal range of interval of from 3 to 10 min.; at longer intervals the response is diminished by the "staircase effect", which is very well developed in this muscle at intervals of half an hour or over; at shorter intervals the response is diminished by fatigue, which is well-marked at intervals of one minute or less. One stimulus a minute (Fig. 1 B), though already producing fatigue, still allows almost complete relaxation. More frequent stimulation hastens the development of fatigue; stimulation frequent enough to yield a fused response results in fatigue soon enough and severe enough to reduce the response to about one-quarter in a few minutes. Excitation of the kind produced by A.C. is suitable, therefore, for producing phasic contractions, but is incapable of producing the long-sustained contractions characteristic of tonus.

In contrast with the response to A.C., the responses to D.C. (Fig. 1 C) applied for 15 sec. once a minute involve such slow relaxations that the contraction is almost continuous, and builds up by a process of summation which conceals the effects of fatigue, if there be any. Stimulation with D.C. is, therefore, exceedingly suitable for producing long-sustained contractions, but it is quite unsuitable for producing phasic contractions because the high viscosity opposes the full development of tension and because relaxation is too slow.

If it were supposed that the animal availed itself of this property of its muscles, and sustained tonic contractions on the basis of discontinuous nervous activity, such as a stretch reflex, producing effects like those of repetitive stimulation with D.C., it would be necessary at times to interrupt the tonic contraction by rapid movements of the same muscles. Fig. 2 A shows that the slow relaxation following D.C. stimulation can be rapidly interrupted by an A.C. stimulus with its consequent rapid relaxation. If a series of D.C. stimuli have induced the contraction to build up to a sufficiently high value as in Fig. 1 C, an A.C. stimulus usually produces no further contraction whatever, but only a pure relaxation; this is true whether the A.C. be applied for 10 sec., when the relaxation, as will be shown, is due to a reduction of viscosity, or whether the A.C. stimulation be continued indefinitely, when relaxation is due to the reduction of viscosity together with the rapid onset of fatigue which, as mentioned above, follows an attempt to sustain a contraction with this form of

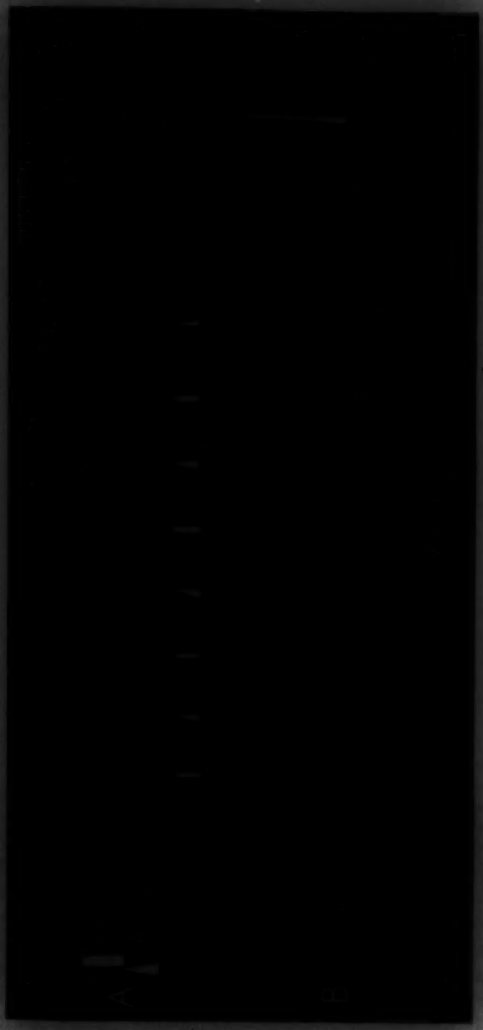

Fig. 2. Mechanical responses of isolated *Mytilus* muscle in sea water at 9·5° C. A. The slow relaxation following a D.C. stimulus (20 sec.) is hastened by interruption of an A.C. stimulus (20 sec., 12 V.). Note that with D.C. stimuli of such long duration the "on" and "off" contractions can be distinguished. B. A series of contractions due to A.C. or D.C., both 12 V., 10 sec. once in 3 min., showing that the responses to A.C. before and after a series of D.C. stimuli are the same, and that the response to D.C. is smaller than that to A.C. but unaffected by the direction of the current along the muscle (arrows indicate the direction).

stimulation. Fig. 2B shows that the D.C. stimuli employed in these experiments were innocuous, the responses of the muscle to A.C. stimuli being the same before and after a series of such stimuli. It also shows that the responses to D.C. stimulation do not depend to any essential degree on the direction in which the current passes along the muscle.

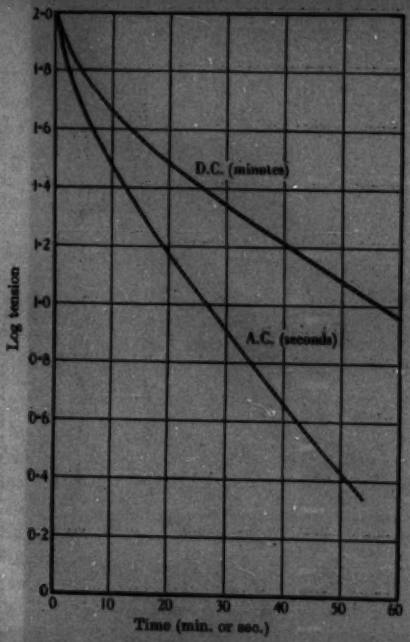

Fig. 3. Isometric relaxation curves after contractions due to stimulation for 10 sec. with D.C. (abscissæ in minutes) and A.C. (abscissæ in seconds). 8·0° C.

The changes of viscosity after cessation of electrical stimuli. The difference between the rate of relaxation of a muscle after an A.C. stimulus and that after a D.C. stimulus is shown quantitatively in Fig. 3. These curves are taken from records of isometric contractions, and correspond with the isotonic records shown qualitatively in Fig. 1A. The isometric relaxation after the A.C. stimulus is 90 p.c. complete in about 30 sec.; and that following the D.C. stimulus in 60 min.—a difference of about 120-fold.

The changes in the viscous-elastic properties of the muscle following contraction due to electrical stimulation last for so long a time that they can readily be studied by recording the isometric stretch and release curves obtained after suddenly adding or removing a load [Winton, 1930].

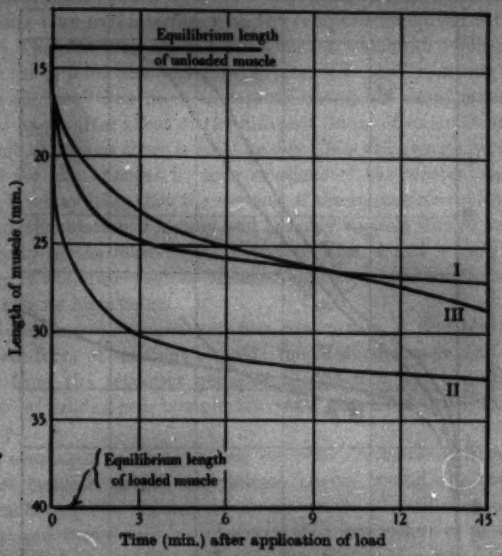

Fig. 4. Isotonic stretch curves following sudden application of a load of 5 g. 8·4° C. Curve I after 4 D.C. stimuli (10 sec.) applied at 3.13 p.m. Curve II after 4 A.C. stimuli (10 sec.) applied at 4.15 p.m. Curve III after 4 D.C. stimuli (10 sec.) applied at 6.0 p.m.

Note. The sudden stretch (undamped compliance) was the same in all three cases; the rate of the subsequent stretch was reversibly hastened by the A.C. stimulation; the final length of the stretched muscle (40 mm.) was the same in all three cases.

Retardation of relaxation after D.C. stimulation might be due either to increase of viscosity, or to the slower disappearance of the tension developed during contraction. The latter alternative is not inherently unlikely, for the tension continues to increase for considerable periods—10 or 15 sec.—after cessation of either an alternating or a direct current, if the duration of the stimulus is relatively short (15 or 10 sec.); a stimulus lasting 30 sec. is usually long enough to produce the maximum tension before it ceases. Which of the two alternatives obtained in the muscle

can be discovered by examining the shape of the stretch and releas
curves following sudden application or removal of weights under isotoni
conditions? A decrease in the compliance of the muscle will result i
curves of the same shape, but with a new length scale reduced in pr

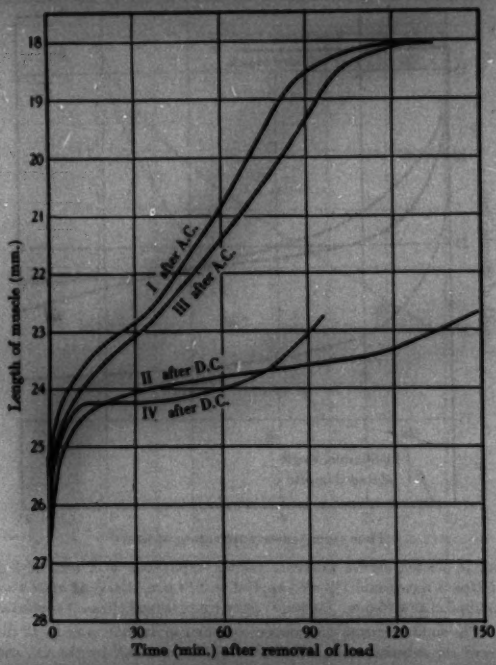

Fig. 5. Isotonic release curves following sudden removal of a load, the remaining load d
to the lever being about 0·2 g. The numbers on the curves represent the order in wh
they were obtained. The stimuli applied were like those in Fig. 4. The rates of shorteni
due to previous A.C. or D.C. stimulation are different in the same sense as are the ra
of stretching (Fig. 4), and the changes are reversible.

portion to the change of compliance which is indicated by the fir
equilibrium length. An increase of viscosity, on the other hand, w
disproportionately extend the time scale, so that both stretch and relea
curves should be slower. The effects of the previous D.C. stimulation a
contrasted with those of previous A.C. stimulation in Figs. 4 and 5. Fig

shows that the relaxation due to application of a load is slower after D.C. stimulation, and Fig. 5 shows that the shortening consequent upon removal of a load is also slower after D.C. stimulation. The curves show that equilibrium, from which the damped compliance of the muscle can be reduced, takes several hours to achieve, and adventitious changes of compliance may well interfere with the comparisons spread over so long a period. The indications from stretch curves, which ran a quicker course because the applied load was much greater than the "restoring force" in the muscle, are that no considerable change in compliance can be detected as an after-effect of the different forms of stimulation; in any case, such change as there is must be much less than would be needed to account for the changes in time relations of the stretch and release curves. We may infer that the slowing of the stretch and release curves after D.C. stimulation is mainly and possibly entirely due to an increase in viscosity of the muscle. The curves in Figs. 4 and 5 show that the viscosity of the muscle can be changed backwards and forwards between its low and its high value.

The undamped compliance after contraction is sensibly the same whichever form of stimulus is used; the *Mytilus* muscle differs in this respect from the retractor penis of the dog, in which a substantial decrease of undamped compliance was associated with increase in viscosity.

The changes of viscosity during electrical stimulation. Although the study of isotonic stretch and release curves provides a method for detecting changes in viscosity of muscle which is relatively simple, both logically and technically, no observer with experience of plain muscle will be entirely happy in relying on the results of a procedure which involves the sudden jerks due to application or removal of loads. Many plain muscles respond to such jerks by contracting, and although my observations on the retractor penis and the *Mytilus* retractor have never been complicated by the appearance of such a contraction, it is possible that the jerks themselves initiate changes in viscosity. The method is in any case not suitable for studying the changes in the muscle during a contraction, for the rapid onset of fatigue renders the period available too short to yield stretch or release curves long enough for safe interpretation.

To overcome these difficulties, another method of analysing the viscous-elastic properties of muscle was developed. The muscle was fixed at its lower end, while the upper end was attached to a helical steel spring. The end of the spring remote from the muscle was attached to the

crank of a Brodie pump, and consequently was submitted to a motion which was approximately sinusoidal. The amplitude of the movement could be varied by the control on the pump, and the frequency could be varied by driving from a synchronous motor through a six speed gear box, with ratios available by turning a knob of 1 : 1, 1 : 2, 1 : 4, 1 : 8, 1 : 16, and 1 : 32. The movement of the junction of the muscle and the spring was recorded; the ratio of this amplitude to that applied to the upper end of the spring depends on the relative compliances of the muscle and the spring. If the muscle has the high compliance of a very weak spring, the two amplitudes will be almost the same. If the muscle is relatively inextensible, its changes of length will be relatively small. Between these limits, the ratio of the two amplitudes depends not only on the relative compliance of spring and muscle, but also on the frequency of the movement; for, as the frequency is increased, the resistance of the viscous components of the muscle to the increasingly rapid change of length becomes greater, and the change of length of the muscle, therefore, becomes less. If the frequency is high enough, the viscous components undergo movements which are negligible compared with the movement of the undamped elastic component. The ratio of the amplitude is then determined entirely by the ratio of the compliances of the undamped elastic component of the muscle and of the steel spring. This range of frequencies can be recognized by the fact that further increase of frequency produces no change in the ratio of the amplitudes. For the *Mytilus* muscle a frequency of 2 per sec. is conveniently within this range. At the other extreme, we can imagine a frequency so low that the amplitude becomes maximal, depending on the ratio of the combined damped and undamped compliances of the muscle to that of the spring. The curves in Fig. 5 would suggest that such frequency would be of the order of 1 in 10 hours, the higher the viscosity of the muscle, the lower the frequency needed to enable the muscle to approach its equilibrium relation between tension and length throughout the cycle.

At an intermediate frequency, the amplitude of the muscle will be intermediate; the ratio of this amplitude, at a frequency of, say, 1 in 2 min., to the minimal amplitude at, say, 2 a second, will depend largely on the viscosity of the muscle; the higher the viscosity, the smaller will be the difference in the amplitude at the two frequencies.

The present series of experiments was concerned with changes in compliance and viscosity of the muscle during contraction, and since, owing to fatigue, the effective maximum duration of a contraction is only a few minutes, only the relatively higher frequencies could be em-

ployed. Fig. 6 illustrates the changes of amplitude of a resting unstriated muscle at different frequencies, when the amplitude applied to the spring was constant. It shows that the variations of amplitude over the relevant range of frequencies are measurable but not large. Fig. 7 shows the amplitude at two frequencies, one almost high enough to give the minimal amplitude even in the stimulated muscle, and the second a

Fig. 6. The effect on the amplitude of movement of a resting *Mytilus* muscle of applying a sinusoidal stretch of constant amplitude through a steel spring at the different frequencies denoted by the numbers at the top of the record (cycles per min.). Time marker 10 sec.

Fig. 7. The effects of alternating current stimulation (3·5 V., 4 per sec., duration given by lower signal) on the amplitude of movement of the muscle when the indirectly applied sinusoidal movement was at a high (20 c.p. min.) and a low (0·6 c.p. min.) frequency. The ratio of the amplitudes at the two frequencies was about 1 : 1·1 in the unstimulated muscle, even if the tension was increased by mechanical stretching, as toward the right end of the record, but it was 1 : 2·3 in the stimulated muscle. Time marker 30 sec.

relatively low frequency. It will be seen that whereas in the unstimulated muscle the amplitude at the low frequency was about 10 p.c. greater than that at the high frequency, in the muscle stimulated with A.C. the amplitude at the low frequency was about 130 p.c. greater than that at the high frequency.

In Fig. 8 the variation of amplitude with frequency is shown in a muscle during stimulation with alternating current, and in the same muscle unstimulated. The relations are qualitatively of the form which would be expected in a system with undamped and damped compliances in series, as implied by the muscle model mentioned above, and the

difference between stimulated and unstimulated muscles shows that a contraction during stimulation with A.C. involves a reduction of viscosity. Contraction during stimulation with D.C., on the other hand, is associated with a viscosity which remains at the intermediate value found after cessation of an A.C. stimulus [Winton, 1935].

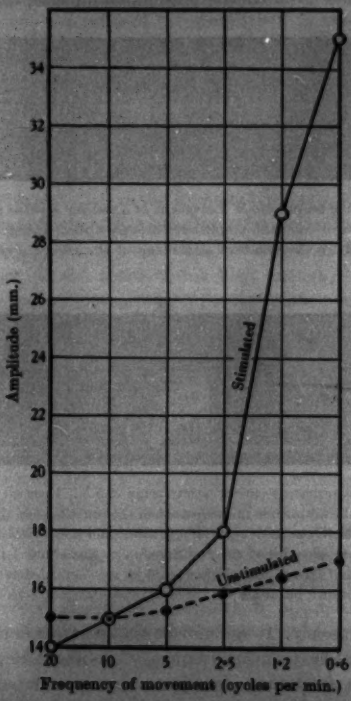

Fig. 8. Curves relating the frequency of the indirectly applied sinusoidal movement of constant amplitude, and the amplitude of movement of the muscle, unstimulated, and stimulated with current alternating at 4 cycles per sec. 3·5 V. 16·2° C.

The significance of frequency of stimulation. Contrasts in the effects on the muscle of direct and alternating currents raise questions such as (1) what frequency of stimulation results in maximum tension development and minimum viscosity, (2) do unidirectional pulses of current

raise or lower the viscosity, (3) what are the relations between the effective frequencies of alternating currents and of interrupted unidirectional currents, and (4) using interrupted unidirectional currents, what is the proportion of the cycle which must be occupied by the current flow if its effects are to approach those of continuous current, and does this proportion depend on the frequency?

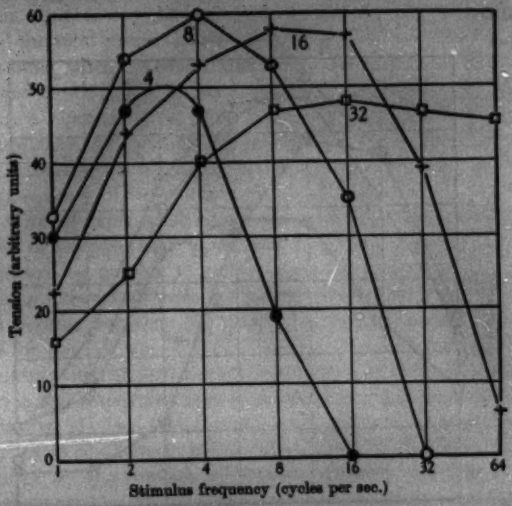

Fig. 9. The relation between the tension developed and the frequency of an alternating current stimulus of 10 sec. duration. The stimulating voltage was constant for any one curve, and proportional to the number on the curve. Note the marked increase of optimum frequency with increase in voltage.

Stimulation with 50 cycle A.C. derived from the mains shows that the optimum voltage is substantially independent of the duration of the stimulus, but that for long continued stimuli (10–20 sec. or more) voltage in excess of the optimum produces actually less tension than the optimum voltage. The rate of relaxation of the muscle, and presumably, therefore, the viscosity, depends on the tension developed in the contraction being quicker the greater the tension. This is true when the contraction is submaximal in virtue of the voltage being too low, and also when it is submaximal in virtue of the voltage being too high.

As sinusoidal currents with a frequency range of 1–100 c.p.s. are not easy to produce, the influence of frequency was studied by stimulating with a commutator which gave rectangular pulses of current; during the first quarter cycle current flowed one way and during the third quarter cycle it flowed the opposite way. No marked differences between the effects of stimulating with the commutator at about mains frequency and those of stimulating with sinusoidal current of the same frequency were noticed.

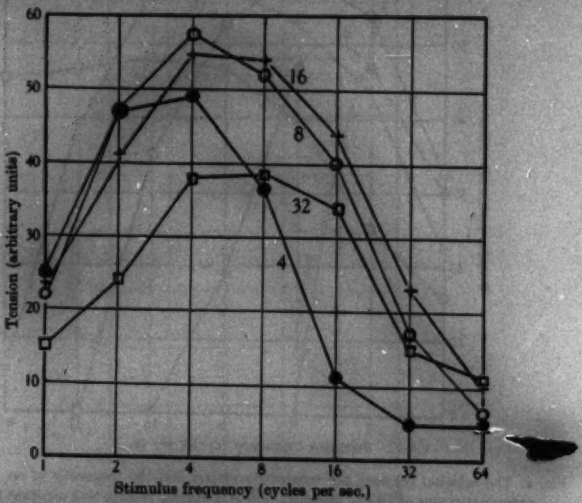

Fig. 10. The relation between the tension developed and the frequency of an intermittent unidirectional current stimulus of 10 sec. duration. The stimulating voltage was constant for any one curve, and proportional to the number on the curve. Note that the increase of optimum frequency with increasing voltage is much less marked than with alternating current stimulation (Fig. 9).

The influence of frequency of such an A.C. stimulus is shown by the typical experiment charted in Fig. 9. In this experiment the duration of each stimulus was 10 sec. The points on any one curve correspond with the same stimulating voltage, and a single curve, therefore, represents the effects of varying frequency when the total stimulating current is kept constant. Errors due to drift in the properties of the muscle were minimized (*a*) by taking a series of observations at each voltage first in

ascending and then in descending orders of frequency, and (*b*) by repeating these series, which had been taken in ascending order of voltages, in descending order of voltages. The mean of the four values so obtained is shown as one point on a curve (Fig. 9). The curves show that the optimal frequency increases markedly with increase in voltage.

Intermittent unidirectional current is readily obtained from the same commutator by disconnecting the circuit which gives the reverse pulse of current. In this way the current flows during one-quarter of a cycle only, and the total current passing in a given time is, therefore, one-half of that passing when the connexions are for alternating current as in the experiment shown in Fig. 9. Except for this, the experiments, of which Fig. 10 is a representative, were performed in exactly the same way. They show that the optimum frequency of intermittent unidirectional current stimulation is much less dependent on the voltage than is the optimal frequency of an alternating current stimulus.

The simplest explanation of the existence of an optimum frequency would be to suppose that it resulted from two opposing tendencies: (*a*) an increasing number of pulses increasing the response, and (*b*) a diminishing interval between the pulses reducing their effect when the interval became less than the relative refractory period. This would account for the increase of optimum frequency with increased voltage of alternating current, but it is difficult to see how it can account for the contrast in this respect between alternating and unidirectional pulses as shown in Fig. 11. This phenomenon could, perhaps, be more profitably summarized by a scheme in which each element of excitation was due to the passage of ions through a particular distance, the ions having to retrace their path before the next element of excitation could begin. The velocity of the ions would depend on the potential gradient, or in the absence of an applied potential, on diffusion. The optimum frequency in alternating current stimulation should, therefore, be roughly proportional to the voltage, because up to this frequency the ions would reach their goal more quickly the higher the voltage, and the greater the total number of elements of excitation the greater the response; beyond the optimum frequency, however, ions could not move far enough to excite before the pulse of reverse current drove them back. In an alternating current field the forward and backward movement of the ions would take about the same time. In an intermittent unidirectional current field, however, the backward movement would depend on diffusion; it would be slow compared with the forward movement, except at the lowest effective voltages, and would be independent of the stimulating

voltages. The speed of the forward movement would, however, depend on the potential gradient as in A.C. stimulation. Hence the optimum frequency should be much less affected by the voltage of unidirectional pulses than by the voltage of alternating pulses (*vide* Fig. 11). We could predict from such a scheme that if the time for back diffusion of the ions were curtailed by allowing the unidirectional pulses of current to occupy one-half a cycle instead of one-quarter, the optimum frequency should be lower. That this is so is shown in Fig. 12.

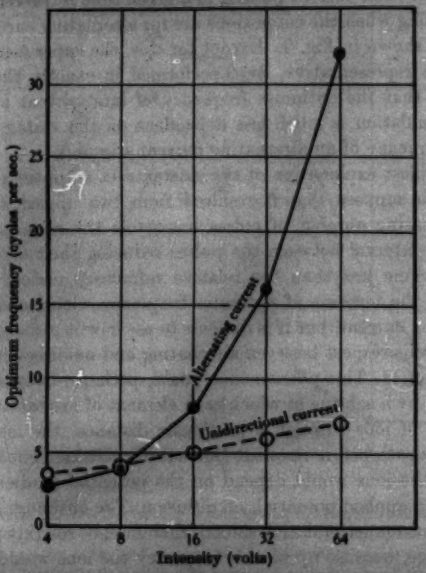

Fig. 11. The relations between optimum frequency and intensity of alternating current and intermittent unidirectional current stimuli of 10 sec. duration in the anterior retractor of *Mytilus*. Sea water 10° C.

The changes in viscosity of muscles due to the different forms of stimuli described in this section have only been investigated provisionally by taking the rate of relaxation as a qualitative guide to the viscosity. The results may be summarized by saying that the rate of relaxation of a muscle is greatest after a maximal contraction, and is lower where for any reason the contraction is smaller. For example, stimulation with

unidirectional pulses (one-quarter cycle) results in about the same relaxation rate as stimulation with alternating pulses, when the voltage and frequency of the stimuli are so chosen that the tension development is the same. This point is illustrated in Fig. 12, in which the broken

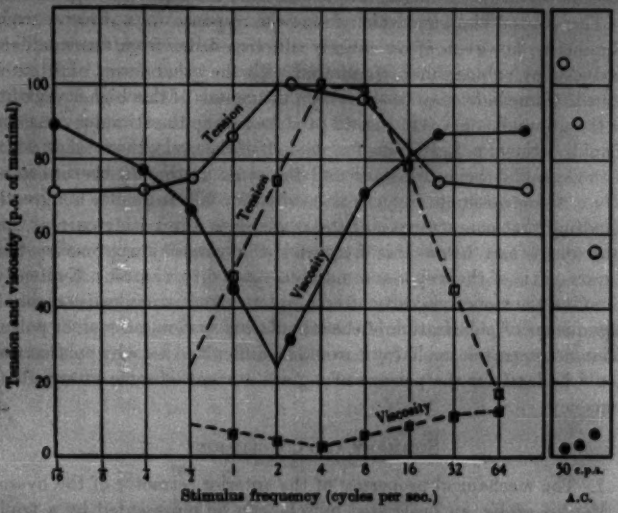

Fig. 12. The effects of frequency of intermittent unidirectional current stimuli (10 sec.) on the tension developed and the relaxation rate (viscosity) of *Mytilus* muscle. The continuous lines correspond with currents occupying one-half of the cycle, the broken lines with currents occupying one-quarter of the cycle. Note that the optimum frequency for the latter stimuli is higher than that for the former; the frequency for maximum tension is the same as the frequency for minimum viscosity.

The column on the right of the figure indicates tensions and viscosities produced by stimulating with mains alternating current (50 c.p.s.) of three different voltages; it is included to show that the relation between tension and viscosity is much the same in alternating current stimulation as in stimulation with unidirectional pulses (one-quarter cycle) of current.

curves show the relaxation rates and tension developments at various frequencies of unidirectional (one-quarter cycle) current stimulation. The relation between relaxation rate and tension development is about the same as that shown in the right-hand column of this figure, where the results of stimulating with sinusoidal currents of 50 c.p.s. are given, the tension development being varied by variation of voltage. This seems to

indicate that there is a fundamental connexion between the magnitude of the mechanical response, manifested as tension or shortening, and subsequent viscosity of the muscle, within the range of conditions that can be produced by alternating or unidirectional (one-quarter cycle) currents of various voltages, frequencies, and durations.

The rise of viscosity which follows a response to a direct current stimulus is, however, of an entirely different order from the small rise produced by submaximal stimulation with the other forms of stimulus named. Some light may be thrown on the genesis of this higher viscosity by the unbroken curves in Fig. 12 produced when the stimulus consisted of unidirectional pulses lasting for one-half of the cycle, the total duration of the stimulus being constant and the same as that in the unbroken curve. At the optimum frequency the viscosity falls to a value not greatly exceeding that characteristic of the response to alternating current, but both above and below this frequency the viscosity approaches that characteristic of the response to uninterrupted direct current. Evidently, therefore, the rise of viscosity after a direct current stimulus is no simple consequence of polarization of the stimulating system, or even of polarization in the muscle itself, for it would be difficult to see why polarization should be least for the passage of a given current at some intermediate frequency.

Summary and conclusions

1. The mechanical properties of the anterior retractor of the byssus of *Mytilus edulis*, an unstriated muscle, can be represented by a triple viscous-elastic model as can those of the retractor penis of the dog.

2. Analysis of isotonic stretch and release curves shows that after cessation of an alternating current stimulus the viscosity of the *Mytilus* muscle is much lower (e.g. 100-fold) than it is after a direct current stimulus. The high viscosity due to the latter persists for an hour or two, but can be readily reduced by stimulation with alternating current.

3. These results are confirmed by observations on the changes in tension in muscles submitted to sinusoidal changes of length at different frequencies. This technique has shown further that the viscosity during A.C. stimulation is greatly lower even than it is after A.C. stimulation, but that the viscosity during D.C. stimulation is not very different from that after A.C. stimulation.

4. The tension development due to a single maximal A.C. stimulus is greater than that due to a single maximal D.C. stimulus, presumably owing to the low viscosity. A.C. stimulation produces the powerful type

of contraction followed by quick relaxation suited to rapid movement. D.C. stimulation for a few seconds every minute or two results in a contraction which is fused owing to the high viscosity and powerful owing to summation; such sustained contraction is relatively immune from fatigue and shows how an unstriated muscle may develop one form of tonus with great economy although the exciting agent is discontinuous as it presumably would be if under nervous control.

5. In a preliminary survey of the relations between frequency and voltage of alternating and intermittent unidirectional stimulating currents, the optimum frequency of A.C. stimuli of constant duration, which is the same for maximal tension and for minimal viscosity, is shown to increase markedly with increase in intensity, that of intermittent unidirectional stimuli less so. With the latter, when current passes during only one-quarter of the cycle the effects on the viscosity are much like those of A.C. stimulation, that is, the greater the tension development the lower is the associated viscosity; when the unidirectional impulses of current occupy as much as one-half of the cycle the viscosity is still fairly low at the optimum frequency for tension development, but at frequencies both above and below this, the viscosity is high and approaches the value due to stimulation with continuous current.

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PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
17 *October* 1936

The relation between the *p*H of the blood and the law of isodynamic equivalence. By F. Kane and J. M. O'Connor

When an isodynamic amount of carbohydrate is substituted for fat in metabolism there is a fall in oxygen consumption and an increase in carbon dioxide production. The increased CO_2 production would be expected to cause, and to require for its removal by the lungs, a fall in blood *p*H. If the process of oxidation be due to the influence of a catalyst *activating oxygen*, and if the power of this catalyst be altered by change in reaction in such a fashion that the fall in *p*H produced by the increase in CO_2 production results in a diminution of the available activated oxygen, the change-over from fat to carbohydrate might result in the isodynamic equivalence effect appearing merely as a consequence of this property of the catalyst.

If this be the explanation of the law of isodynamic equivalence it should be possible to demonstrate (*a*) that artificially produced changes in the *p*H of the blood plasma cause proportionate changes in oxygen consumption; and (*b*) that under normal conditions the *p*H of the blood plasma falls with rising respiratory quotient by an amount sufficient to account for the fall in oxygen consumption required by the isodynamic law.

Experiments have been done on these two questions:

(A) Determinations of oxygen consumption have been made on rabbits anæsthetized with urethane and maintained at approximately constant temperatures. Curare was given when necessary to suppress shivering. The reaction of the blood was altered by addition of CO_2 to the inspired air, or by injections of $NaHCO_3$ or HCl. The *p*H of the plasma was determined from its CO_2 content and the partial pressure of CO_2 in the alveolar air.

As there is reason to believe that different catalysts control oxidation at different temperature ranges, three sets of observations were made at

different temperatures. At 37° C. and at 32° C. the oxygen consumption rises to *p*H of about 7·5 by about 5 p.c. per 0·1 *p*H. At 25° C. *p*H has no influence on oxygen consumption between 7 and 8.

As the energy equivalent of oxygen for carbohydrate is about 7 p.c. greater than for fat the theory requires a fall in *p*H of the order of 0·1 in passing from an R.Q. of 0·7 to 1·0 at normal body temperature.

(B) The normal *p*H in man varies by about 0·1. Food produces approximately corresponding alterations in the partial pressure of the alveolar CO_2. It is shown that changes in the partial pressure of CO_2 in alveolar air are associated with alterations in R.Q. in the direction and approximately to the extent required by theory.

Potentiation of adrenaline activity.

By D. Burns and J. Secker

Burns & Watson [1920] reported that guanidine salts in doses too small to affect mammalian blood pressure prolonged the pressor action of adrenaline. Recently Bacq [1936], using the denervated nictitating membrane of the cat as a test object, showed that "certain substances which, *in vitro*, protect adrenaline from oxidation" (e.g. pyrogallol) potentiate and prolong its action *in vivo*.

Using Folin's colorimetric method, it can be shown that 1γ of guanidine carbonate per ml. of shed whole blood delays the destruction of added adrenaline (or phenols in general) so that after 1 hour at 22°, 75 p.c. of the adrenaline remains as against 52 p.c. in the control. This protective action of guanidine differs from that of pyrogallol, etc. The latter substances presumably act more avidly than adrenaline as oxygen acceptors and lower the oxidation potential. On the other hand, guanidine is a very stable substance withstanding any ordinary means of oxidation [Burns, 1916]. Either guanidine directly interferes with the oxidative system in shed blood by inhibiting enzyme action, or indirectly, by increasing the proportion of adrenaline protected by enclosure within the red cells [Bain *et al.* 1936]. Experiments by Meyerhof [1921] which demonstrated the depression of yeast fermentation by guanidine salts might be considered to lend weight to the first possibility, but his results could be explained on the second hypothesis, i.e. action on the cells. Guanidine undoubtedly influences the uptake of solutes by erythrocytes [Secker, 1925] and decreases the intake of oxygen by blood and by

tissue slices [Banu & Gavrilescu, 1934]. A trace of guanidine markedly retards the auto-reduction of HbO_2.

In vivo, experiments show that the inactivation of adrenaline is slowed down in the presence of guanidine salts as indicated by the prolongation of its pressor activity and its action on the nictitating membrane. Further, guanidine so acts on the sympathetic receptive complex that the tone of the whole sympathetic system is raised to such an extent that parasympathetic irritability is reduced, as shown for the blood-sugar level by Clark [1924] and for the vaso-motor system by Burns & Secker [1935].

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The initiation of all-or-none responses in muscle by acetylcholine. By S. L. Cowan

Evidence has already been advanced [Cowan, 1936*a*, *b*] that in frog nerve-muscle preparations, provided that the accommodation of the muscle has a high enough value, each amount of the chemical transmitter liberated at the nerve endings in response to a single volley in the nerve trunk acts locally on the muscle fibres to initiate an all-or-none response. Brown *et al.* [1936] have presented evidence that in mammalian preparations the acetylcholine [ACh.] liberated at motor nerve endings produces all-or-none responses in the muscle. The question arises: How does acetylcholine initiate all-or-none responses in muscle fibres?

Riesser & Steinhausen [1923] and Schäffer & Licht [1926] have reported that the localized application of a solution containing 1.10^{-5} ACh. to toad muscle produced a monophasic potential change which had a maximum value of about 1 mv. and which lasted about 1½ min. Kruta & Paulian [1935] have made similar observations on frog

muscle. In all these experiments it seems unlikely that the actual concentration of ACh. applied to the surface of the muscle fibres ever reached the value of 1.10^{-3}.

The effect of ACh. chloride on the injury potential of frog's isolated and prostigmin-treated (3.10^{-6}) sartorius muscle has been examined. The tibial end of the muscle was injured by burning, then after the potential had attained a steady value (40–70 mv.), prostigmin-Ringer's fluid surrounding the pelvic end was replaced by fresh prostigmin-Ringer's fluid containing 1.10^{-3} ACh. chloride. The injury potential fell immediately by about 35 mv., and then, in the 40 min. following, returned to its original value approximately in an exponential manner. Control experiments with Ringer's solution in which part of the sodium chloride had been replaced by potassium chloride showed that 0·5 p.c. of KCl could also produce a depolarization of 35 mv., but in this case the depression developed more slowly and there was no recovery in 40 min.

In other experiments with prostigmin-treated muscle neither choline nor sodium acetate, each in concentrations up to 0·5 p.c., was found to affect the resting potential.

Curare 1.10^{-5}, equivalent to about 2.10^{-6} curarine chloride, was without influence on the injury potential of prostigmin-treated muscle, but when the pelvic end of the curare-treated muscle was tested with ACh. its threshold to depolarization was found to have been raised considerably. 1.10^{-5} ACh. produced a depolarization of only 6 mv.

The experiments described here suggest that in nerve-muscle preparations the ACh. liberated at the nerve ending initiates a propagated response in a muscle fibre by producing a local depolarization, and since the hydrolysis products of ACh. have been found inactive such a depolarization would disappear along with the ACh. Measurements of the minimum change in the local potential necessary to set up a propagated response in a muscle fibre do not yet exist. From the experiments of Schmitz & Schaefer [1933; also Schaefer, 1934] on electrotonus in nerve it might be inferred that a change of about 10 mv. would be sufficient. If it is justifiable to extend this inference to muscle, then it appears that the amounts of ACh. used by Brown *et al.* in their "short range" arterial injection experiments would be sufficient to give the required depolarizations. The action of curare in raising the threshold of muscle to depolarization by ACh. would explain why curarized muscle is no longer excited on stimulation of the nerve although ACh. continues to be liberated from the nerve endings [Brinkman & Ruiter, 1924; Dale *et al.* 1936].

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Synergism and antagonism of acetylcholine and quaternary ammonium compounds. By J. Raventos. (*From the Department of Pharmacology, University of Edinburgh*)

The action of the series $Me_3N.R.Br$ was studied. The concentration of acetylcholine needed to produce 50 p.c. inhibition of the mechanical response of a strip of frog's auricle was measured and the change in this concentration produced by the addition of quaternary ammonium salts was determined. The action of the latter compounds when administered alone also was measured. Members of the series from $Me_3N.Me$ to $Me_3N.Am$ produced depression of the auricle, and this effect was additive to the action of acetylcholine. These actions increased steadily from $Me_3N.Et$ to $Me_3N.Bu$; $Me_3N.Me$ was more powerful than $Me_3N.Et$ and $Me_3N.Am$ was much weaker than $Me_3N.Bu$. On the other hand $Me_3N.Oct$ and $Me_3N.Ce$ produced no depression of the auricle and acted as powerful antagonists to acetylcholine. These compounds therefore resemble the members of the series $Et_3N.R$, whose "atropine-like" action was described and measured by Külz [1923].

The action of curarine also was tested; this drug produced no depressant action on the auricle at a concentration of 1 in 10^4, but this concentration acted as a powerful antagonist to acetylcholine.

The same general effects were observed with the rectus abdominis. Acetylcholine and the lower members of the series $Me_3N.R$ produced contracture whilst $Me_3N.Oct$ and curare produced little and no contracture respectively, but antagonized the actions of acetylcholine and of the lower members of the $Me_3N.R$ series.

Külz [1923] and Ing & Wright [1931] agree that $Me_3N.Oct$ is approximately equal to $Me_3N.Me$ in respect of its power to paralyse motor nerve endings. The sharp change observed between the butyl and

octyl compounds as regards the "muscarin-like" action of the $Me_3N.R$ series therefore does not occur in the case of their action on motor nerve endings. The pharmacological actions of $Me_3N.Oct$ closely resemble those of curarine, not only in regard to its depressant action but also in regard to its power to antagonize acetylcholine in the frog's heart and muscle.

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Standardization of prolactin. By F. J. Dyer.
(*From the College of the Pharmaceutical Society, London*)

Riddle *et al.* [1933] have tested the potency of different extracts of prolactin prepared in their laboratory, by weighing the crop glands of ring-doves and immature pigeons which received daily injections for several days. They defined a "unit" as the activity contained in 1 mgm. of one of their isoelectric precipitates.

I have found that preparations of the hormone may be assayed upon mature pigeons, irrespective of sex, age or body weight, by using groups of six birds and injecting the preparation into their pectoral muscles three times daily for 4 days. On the 5th day the birds are killed, the glands dissected, and after pressing the glands between filter paper they are weighed to the nearest centigram. In each experiment four groups of six birds were used, three groups being given 0·25, 0·5 and 1·0 c.c. daily per bird, and the fourth group, no dose. In Table I the mean results of

Table I. Response of crop glands of pigeons to prolactin
(Summarized results of four experiments)

No. of birds in group	Daily dose of prolactin (c.c.) (for 4 days)	Average weight of two glands (gm.)
25	No dose	0·92
12	0·25	2·23 ±0·34
24	0·5	3·49 ±0·33
12	1·0	4·18 ±0·20
		[Average ±0·29 or 10 %]

four of these experiments are given. The standard deviation of the mean weight of glands from twelve birds is about 10 p.c. When the average weight of the glands for each group was plotted against the actual dose of the preparation, a smooth curve was obtained. When the average weight of crop gland was plotted against the logarithm of the dose, the points

lay on a straight line, and the equation to the regression line through the points was $y = 3{\cdot}9x + 0{\cdot}7$, where x is the log of the dose and y is the average weight of the two crop glands in a group of six pigeons (gm.).

A better response is obtained by dividing the daily dose into three rather than into two fractions. In a later experiment in which twenty-four birds were used, a good response was obtained after 2 days, and probably a maximum response after 3 days. A milky secretion began to appear between the 3rd and 4th days.

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PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
14 *November* 1936

Correlation of observations on the sugar production of the livers of normal and diabetic fasting dogs. By CHARLES REID

The observations in the following statement are based on data derived from fasting dogs.

By different techniques Mann [1927] and Wierzuchowski & Fiszel [1935] have brought forward presumptive evidence that the average glucose utilization of the tissues of normal dogs with normal blood glucose is of the order of 0·25 g./kg. body weight/hour (i.e. 60 g. per diem for a 10 kg. dog), and Yater *et al.* [1933] have shown that the corresponding figure for depancreatized dogs is only about 0·19 g. (i.e. 45 g. per diem for a 10 kg. dog) [cp. Young's [1936] calculations *re* sugar formation from fat]. Moreover, the B.M.R. is not lowered by pancreatectomy and, as fasting depancreatized 10 kg. dogs excrete 10–15 g. glucose per diem, the glucose production of their livers appears to be of the same order (i.e. 45 + (10–15) g.) as the normal.

Normal fasting 10 kg. dogs excrete 2–3 g. N per diem, whereas depancreatized animals of similar weight excrete 4–5 g. N [Lusk, 1928]. This indicates an approximate excess catabolism of protein to the value of 50 cal. in depancreatized animals, which, on the other hand, lose glucose in the urine to the value of 40–60 cal. No account is taken of the small energy value of the extra ketones excreted in the urine of fasting depancreatized animals.

Depending on the accuracy of the above data and on the presumed justification of correlating average data from different animals, the following deductions may be made:

(1) Compared with normal fasting animals, there does not appear to be evidence of an actual increase in glucose production by the liver of fasting depancreatized animals.

(2) The excretion of glucose in diabetes during a fast indicates an excess production of glucose by the liver relative to the capability of the tissues to deal with it.

(3) In contrast to normal fasting animals, energy is lost during fasting in pancreatectomy diabetes by the excretion of glucose, but extra catabolism of an isodynamic quantity of tissue protein has occurred.

(4) In metabolic studies on diabetes during fasting stress should be laid on the apparent isodynamic values of the glucose lost and the extra protein catabolized rather than on a D/N ratio based on the total N excretion.

(5) The protein catabolism of fasting in depancreatized animals is greater than that in normal fasting animals, probably because the tissues of the latter can use more glucose and so their protein catabolism is partly spared.

In connexion with deductions (3), (4), (5), it is of interest to observe that, when isodynamic quantities of (*a*) fat, (*b*) fat and carbohydrate, or (*c*) carbohydrate are fed to non-emaciated fasting normal animals, fat alone does not diminish the excretion of N; fat and carbohydrate will do so, but the largest decrease is got when carbohydrate alone is given [Lusk, 1928]. On the other hand, in the non-emaciated fasting diabetic organism, the excretion of N is not diminished by (*a*), (*b*) or (*c*). This may suggest that, in the normal organism, part of the protein catabolism is spared by the use of extra glucose (as compared with the diabetic) in the presence of endogenous insulin so that tissue repair can keep pace with wear and tear. This mechanism appears to be lacking when the supply of endogenous insulin is stopped [cf. Reid, 1936].

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The source of the histamine-like constituent of blood. By C. F. Code. (*From the National Institute for Medical Research*)

I have been investigating the distribution, between the cellular constituents of shed blood and the plasma or serum according to the conditions, of the substance, acting like histamine, which can be extracted by methods such as that of Barsoum & Gaddum [1935]. I have used a

method of extraction which is a modification of this latter, and the details will be separately published. When separation was made by the centrifuge, it early became clear that the active substance was mainly obtainable from the cellular layer when the blood was prevented from clotting, the extract from the clear plasma being very weak; but that if blood was allowed to clot, or whipped, the activity largely passed into the serum. Finer conditions of separation of unclotted blood have shown that the red cells, like the clear plasma, yield hardly any activity to extraction, and that the main, if not the only, source of the active substance is to be found in the layer containing the white cells and platelets. I hope to make a closer differentiation, as between leucocytes and platelets, and, with the aid of this clue, to attempt a nearer approach to the isolation of the active substance.

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Adrenaline dilatation. By R. J. S. McDowall and I. McWhan

In a previous communication it was pointed out that it is possible to obtain a dilatation of vessels with histamine and adrenaline in a dilution of less than 1–10,000,000 in cats' limbs perfused with alkaline calcium-free Ringer's solution. The adrenaline dilatation is often slight at a time when histamine dilatation is well marked. It is present in rats but not in rabbits. Dilatation by adrenaline is abolished by atropine; a result which is all the more unexpected as atropine is not known to influence the fall of blood pressure caused by small doses of adrenaline in cats under ether anæsthesia, and possibly of interest in regard to humoral transmission.

Since the experiments were carried out, it has been found that Schmidt [1921] and Wehland [1924] have obtained similar results in frogs, while Kolm & Pick [1920] have described what they call a "vagotropic" action of adrenaline.

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